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Edited by

I. Geller, W. C. Stebbins and M. J. Wayner

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Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function

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Volume 1, Supplement 1, 1979

This Supplement to Volume 1 of *Neurobehavioral Toxicology* contains the Proceedings of the Workshop on "Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function," which was held April 1–4, 1979, at the Hilton Palacio del Rio Hotel in San Antonio, Texas. This meeting was organized by the Southwest Foundation for Research and Education with the direction of Dr. Irving Geller; and was sponsored by the United States Environmental Protection Agency, Office of Toxic Substances, under Contract No. EPA 68-01-4870. These Proceedings serve as the Final Report No. EPA 560/11-79-010. The opinions expressed in these articles, however, are those of the authors and are not necessarily endorsed nor shared by the Environmental Protection Agency.

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Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function

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San Antonio, Texas, April 1–4, 1979

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Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function

Supplement to ***NEUROBEHAVIORAL TOXICOLOGY***

CONTENTS

Preface. GOULD, D. H. 1

Opening Remarks. PAGE, N. P. 3

Introduction and Overview. GELLER, I. and W. C. STEBBINS 7

Use of discrimination behavior for the evaluation of toxicants. GELLER, I., E. GAUSE, R. J. HARTMANN and J. SEIFTER 9

Effects of toxicants on visual systems. MERIGAN, W. H. 15

Effects of toxicants on the somatosensory system. MAURISSEN, J. P. J. 23

Comparative behavioral toxicology. STEBBINS, W. C. and D. B. MOODY 33

Trialwise tracking method for measuring drug-affected sensory threshold changes in animals. ANDO, K. and K. TAKADA 45

Motor activity: A survey of methods with potential use in toxicity testing. REITER, L. W. and R. C. MACPHAIL 53

Reinforcing properties of inhaled substances. WOOD, R. W. 67

Behavioral assessment of risk-taking and psychophysical functions in the baboon. BRADY, J. V., L. D. BRADFORD and R. D. HIENZ 73

Operant conditioning of infant monkeys *(Macaca fascicularis)* **for toxicity testing.** RICE, D. C. .. 85

Effects of pre- and post-natal lead on affective behavior and learning in the rat. FLYNN, J. C., E. R. FLYNN and J. H. PATTON 93

Performance and acquisition of serial position sequences by pigeons as measures of behavioral toxicity. MCMILLAN, D. E. 105

Effects of solvents on schedule-controlled behavior. COLOTLA, V. A., S. BAUTISTA, M. LORENZANA-JIMÉNEZ and R. RODRÍGUEZ 113

Testing for behavioral effects of agents. DEWS, P. B. and G. R. WENGER 119

Some problems in interpreting the behavioral effects of lead and methylmercury. LATIES, V. G. and D. A. CORY-SLECHTA 129

Screening for neurobehavioral toxicity: The need for and examples of validation of testing procedures. TILSON, H. A., C. L. MITCHELL and P. A. CABE 137

Behavioral epidemiology of food additives. WEISS, B., C. COX, M. YOUNG, S. MARGEN and J. H. WILLIAMS 149

Psychological test methods: Sensitivity to long term chemical exposure at work. HÄNNINEN, H. . 157

Quantitative analysis of rat behavior patterns in a residential maze. ELSNER, J., R. LOOSER and G. ZBINDEN 163

Preceding page blank

Comparison of neurobehavioral effects induced by various experimental models of ataxia in the rat. JOLICOEUR, F. B., D. B. RONDEAU, A. BARBEAU and M. J. WAYNER 175

Methodological problems in the analysis of behavioral tolerance in toxicology. BIGNAMI, G. 179

Morphological studies of toxic distal axonopathy. SCHAUMBURG, H. H. 187

Cellular responses to neurotoxic compounds of environmental significance. SPENCER, P. S. 189

Physiological and neurobehavioral alterations during development in lead exposed rats. FOX, D. A. 193

A preliminary test battery for the investigation of the behavioral teratology of selected psychotropic drugs. BUTCHER, R. E. and C. V. VORHEES 207

Assays for behavioral toxicity: A strategy for the environmental protection agency. WEISS, B. and V. G. LATIES 213

Final Comments. SEIFTER, J. 217

Author Index 219

Subject Index 221

Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function
Neurobehavioral Toxicology, Vol. 1, Suppl. 1, p. 1. ANKHO International Inc., 1979.

Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function

Preface

The subject of this Workshop and the desired output is clearly stated in the title. It was held "to obtain a scientific assessment for regulatory decision-making of the currently available methodologies necessary to determine the toxic threat to human health and the environment posed by all chemicals in commerce." The Office of Toxic Substances of the Environmental Protection Agency is required in its enabling act, the Toxic Substances Control Act (TSCA), to carry out such determinations in the area of behavioral disorders, among others. This meeting was therefore initiated with the inspiration of Dr. Joseph Seifter.

When I became Project Officer for this Workshop, I soon realized how fortunate the Office of Toxic Substances was to have Irving Geller as the guiding hand to organize the meeting. It is indeed true to say that the meeting could not have succeeded without his outstanding knowledge of the behavioral field, and his untiring efforts to pull all the loose ends together. With the invaluable assistance of Dr. William Stebbins, he achieved a program which included the best international thinking and research in behavioral toxicology, and gave the EPA its best chance of succeeding in its prescribed responsibilities.

The various opinions I have heard indicate that this Workshop did succeed in delineating areas of agreement. To this extent, EPA can be confident that meaningful tests are available for measuring sensory and functional impairment, and beyond this, changes in the more complex areas of discriminant and learning processes and reproductive behavior. It has, however, clearly been confirmed that the full breadth of behavioral toxicology with its integration of complex interactions and compensatory mechanisms may never be fully circumscribable. In any case, since EPA must take into account the economic realities, it could not prescribe every one of a completely predictive set of tests since there would be so many, even if such a set could be developed.

The answer to achieving adequate guidelines for regulatory consistency may lie in some approaches which merit further exploration. The quantitative activities of various reference compounds in a selection of well-characterized tests treated with multivariate statistical methods may give profiles which delineate different types of neurobehavioral toxicity, and which may be useful in determining the qualitative toxicities of unknowns. Each such profile might then suggest the need for more extensive testing in certain critical areas of behavior. Then, as suggested by Weiss and Laties in the last paper, EPA by consultation with a scientific advisory board could decide whether the sponsor's proposed set of tests properly covers the likely areas of concern in light of the latest understanding of the discipline.

Finally, I must express my thanks for the valuable contributions of the session chairmen and all of the speakers who, of course, were the raison d'être of the meeting. And for this report, thanks again to Irv Geller, Bill Stebbins and Matt Wayner for the efficient editing process which has led so expeditiously to this publication. Only one contribution could not be included in this publication and the final material was received by the Publisher on August 13, 1979.

DAVID H. GOULD
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Opening Remarks: TSCA Requirements for Testing Chemicals for Behavioral Effects and Neurotoxicity

NORBERT P. PAGE

Environmental Protection Agency

ON behalf of the Environmental Protection Agency (EPA) I join with Mr. Goland in welcoming you to this important workshop on Behavioral and Neurotoxicologic Test Methods. I particularly want to thank Dr. Irving Geller, Dr. William Stebbins, and Dr. David Gould for developing such a promising program. The actual need for this workshop was identified over two years ago, Dr. Joseph Seifter of the EPA and Dr. Geller of the Southwest Foundation for Research and Education providing the initiative to organize and enter the workshop into the EPA's program. The need for a successful workshop is even greater today than was realized then.

We have with us many national and international experts in the behavioral sciences and other areas of toxicology, and I look forward to an enlightening discussion of testing methods in behavioral and neurotoxicology. To provide some groundwork for this discussion and at the same time keep introductory remarks brief, my comments will be restricted primarily to three subjects: (1) the regulatory framework within which the workshop results might be utilized; (2) the EPA's responsibilities for testing under the Toxic Substances Control Act (TSCA) and its approach to implementing standards; and (3) the specific objectives or issues for consideration by this workshop.

The overall objective for the human behavioral workshop described in the announcement follows: "The workshop is to obtain a scientific assessment for regulatory decision-making of the currently available methodologies necessary to determine the toxic threat to human health and the environment posed by all chemicals in commerce." I would like to stress one part of that objective—the need for scientific assessment of currently available methodologies. Under TSCA, EPA has the authority and the responsibility to ensure manufacturers provide data on which the assessment of unreasonable risk can be made. Congress singled out several key health effects of concern, one of which was behavioral disorders.

CHEMICALS AS BEHAVIORAL AND NEUROTOXICITY DETERMINANTS

How big a role do chemicals play in the human behavioral disorder problem? Some scientists say their role is minimal. Others claim chemicals play a very major role which generally has gone unrecognized. To me it seems likely that behavioral disorders are the result of a complex array of many factors including genetics, nutritional aspects, our socioeconomic factors, diseases, and of course, chemicals. It is certain that some chemicals play a major role in the etiology of neurological or behavioral disorders. Several come to mind immediately: the role of heavy metals, such as lead and mercury, a number of the pesticides, including many organophosphates, and some of the chlorinated chemicals such as kepone. An important group that has been incriminated is the chlorinated solvents. I am sure as we move through this workshop we will hear of many other chemicals which have been shown to produce behavioral or neurotoxic effects.

METHODS OF ASSESSMENT

How can we assess for the behavioral or neurotoxicology potential of chemicals? One very obvious method is the association of conditions observed in humans with exposure to specific chemicals. Like all epidemiological studies, this approach is expensive to conduct. Moreover, it is difficult to sort through subjective complaints where exposures may be to several chemicals, and where many modifying factors interact. Indeed, this is not an easy task. Some papers to be presented in this workshop will deal with this aspect of human observation and surveillance. The other main category of assessment methods is the whole animal tests of various types. These range from general toxicity studies where behavior or neurological effects may be observed as a part of a routine health examination to the more sophisticated and specific or tailored tests which are designed to assess behavioral and neuromotor function in a more consistent manner.

REGULATORY FRAMEWORK FOR TSCA, SECTIONS 4 AND 5

The regulatory framework of TSCA is unique for Federal legislation in that it provides specific authority in Sections 4 and 5 to require manufacturers or processors to provide data to EPA for assessment purposes. Section 4 pertains to existing chemicals or categories of chemicals; whereas Section 5 pertains to new chemicals which are to be manufactured. Under Section 4, EPA issues chemical test rules that define a chemical or categories of chemicals which must be tested, the effect to be tested for, and the standards

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by which the testing will be performed. Under Section 5, the Agency has no specific testing authority; however, a premanufacturing notification must be submitted to EPA 90 days prior to manufacture. In that premanufacturing notification, the manufacturer must provide data on which the Agency can make an assessment of risk to human health and the environment.

The approach we are using with Section 4 is to develop and place into the Code of Federal Regulations generic standards for various health effects. These standards will then be referenced at a later time when chemical test rules are proposed. At the time of proposing a chemical test rule, specific modifications to the generic standards can be made so as to customize the standards to the chemicals or category of chemicals to be tested. Moreover, the standards must be reviewed annually and revised as appropriate to assure their currency with scientific development.

A number of health effects testing standards have been developed and will shortly be proposed in the *Federal Register*. Four of these should be published by the end of April. These are testing for oncogenicity, nononcogenic chronic effects, all chronic effects and good laboratory practices. A number of other health standards should be proposed in the early summer. These are for acute toxicity including lethality, eye irritation, dermal sensitization and dermal irritation, subchronic toxicity testing, teratogenicity, reproductive effects, and mutagenicity. This latter group of standards will be proposed basically as they appear in the guidelines proposed last August for use in registration of pesticides under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA).

We have not made a decision on behavioral or neurotoxicity standards. The reason for this is simple. We are uncertain as to which test systems are well enough validated and acceptable to the scientific community. Therefore, results of this workshop will be carefully reviewed by EPA in making its decision on how to proceed with these particular standards.

COORDINATION WITH U.S. AND INTERNATIONAL ORGANIZATIONS IN DEVELOPMENT OF TEST STANDARDS

The Office of Toxic Substances is aware that EPA is not the only organization which has the responsibility for developing test methods. Within EPA we have joined with the Office of Pesticide Programs to form a joint work group to develop test methods which are basically consistent for the TSCA and for FIFRA. We have also joined with the three other U.S. Federal Regulatory Agencies in forming the Interagency Regulatory Liaison Group (IRLG). The IRLG committee on guidelines and standards is attempting to develop consistent standards and guidelines for EPA, FDA, CPSC, and OSHA. In addition, we are a partner or member of the Organization for Economic Cooperation and Development (OECD). The OECD will propose test standards for use by the international community in the testing of chemicals for toxic chemical control. The EPA is attempting to harmonize our test standards with the OECD.

SPECIFIC BEHAVIORAL OR NEUROTOXICOLOGICAL STANDARD

I would like now to turn my attention to specific behavioral or neurotoxicological standards that have been proposed or are under development. Under the proposed FIFRA guidlines a very minimal set of specific tests are proposed in this area. They consist of basic observations in the general toxicity tests and two specific tests for delayed neurotoxicity using hens. These are also observational tests, primarily directed to testing organophosphates and esterase inhibitors. Nothing is proposed or planned at this time by the IRLG. The OECD so far has completely avoided discussion of the needs or methods of testing for behavioral effects.

What chemicals should be tested? Of the thousands in the environment, there are probably some "sleepers" that are responsible for some of the bazaar, behavioral or neurological conditions which exist in the human population, but for which a specific cause and effect relationship has not been established. These chemicals must be identified for testing. Since Congress recognized that EPA was not the only Agency that had a concern for proper and useful data generation, it provided a provision for an interagency committee to select chemicals for EPA to test, the Interagency Testing Committee (ITC). This committee is composed for representatives of eight different Federal agencies as well as participating observers from a number of other agencies. The committee is authorized to designate up to fifty chemicals or categories of chemicals for testing at any one time. As of this time, the ITC has designated approximately 25 chemicals and categories of chemicals to EPA.

Few of the designated chemicals are proposed for testing for specific behavioral or neurotoxic effects. Several, however, are proposed for basically a general toxicity profile in which neurologic effects would be one effect to be tested for. A few of those which are proposed, for example acrylamide and arylphosphates, are already known to have neurotoxicology effects. We would welcome the review and comments by the work group participants on the 25 different chemicals or categories. If you do not have this list of chemicals, we would be happy to provide this to you.

As I indicated earlier, under Section 5, there is no provision for specific testing requirements for new chemicals, unless they fall within a category which has been proposed for testing under Section 4. Such a chemical, cannot be manufactured until it has been appropriately tested. The premanufacturing notification under Section 5 will commence 30 days after the publication of the inventory of chemicals currently in commerce. We expect that the inventory will be published in late May or early June, and therefore premanufacturing notification will begin in June or July.

We have little concept at this time as to the amount of testing that will be performed on new chemicals by the manufacturer or the type of test data that we may be provided, especially in the health area. It could be rather minimal and consist primarily of acute toxicity tests, mutagenicity tests and perhaps some subchronic toxicity testing. This will, of course, depend upon the volume of the chemical the manufacturer expects to produce and market, its release into the environment and the anticipated human exposure. In the event that the Agency does not have sufficient data provided on potential health or environmental effects and thus is unable to conduct a meaningful risk assessment, the EPA can undertake legal proceedings to prevent manufacture of the chemical.

In our attempts to impliment TSCA, we recognize that we are pushing the state of the science in developing test standards. No other legislation requires actual standards to be placed in the Code of Federal Regulations. The FIFRA requires that the Agency develop testing guidelines for the registration of pesticides. The FDA reviews pharmaceuticals and food additives but does not have an assigned responsibility to provide standards for test methods to be used for the development of the toxic effects data.

I would like to mention one aspect in relation to the test methods program of the Office of Toxic Substances. We are attempting to provide for the validation of many tests that are in current use or proposed for use. A number of interagency agreements or contracts have been awarded to validate a number of test methods in certain areas, in particular that of mutagenicity or short-term tests, in acute effects and subchronic tests. We have issued a contract "Request for Proposal" to validate several of the promising tests on behavioral or neurotoxic effects. This "Request for Proposal" closes on April 30. I would encourage those of you that have the interest and scientific resources to undertake such a validation program on behavioral or neurotoxicology test methods to obtain a copy of this RFP. It is possible that some of the findings of this workshop will be useful in deciding the nature of the validation program.

ISSUES FOR CONSIDERATION BY THE WORKSHOP

I would like to conclude by proposing a list of issues that I think the workshop should consider during these next two days. These issues are directed toward the three different forms of tests which may be required under TSCA. One of these would be routine observational assessments that can be made during routine or general toxicity tests. Such observations can enhance the quality of information derived for assessment purposes. The second type of testing is the neuropathology, neurophysiology or neurochemistry examinations, which may also be conducted as a part of routine general toxicity tests or perhaps the specifics for those effects. The third type of test include those which are very specific and are designed for behavioral or neurological functions including that on the developing fetus.

The questions to be considered by the workshop are as follows: (1) How can we strengthen the acute, subchronic or chronic toxicity tests to provide the best possible indication of potential behavior of neurotoxicology effects? (2) Can and should we require a greater level of pathology examinations? How sensitive is pathology in detecting behavioral or neurotoxic effects? (3) Can we propose meaningful and validated neurochemical, physiological or neuropathology parameters which can be used in testing for neurologic or behavioral effects? (4) What existing tests for sensory, motor or cognitive effects are well enough developed and validated to be used as standards at this time? (5) Are there tests now in the research stage that need further development and validation? (6) What areas need further research on test methods? (7) How can we best group the various tests to provide for a safety assessment scheme? Should we go with a battery or a sequential scheme, and if so what would be the criteria for choosing the various tests? (For example: use pattern, structure relationships, results of prior tests, production level, etc.)? (8) Is there a logical and scientific scheme which can be used to test for certain classes of chemicals.

These are the kinds of questions OTS must address as it proceeds with its program for developing test standards under TSCA. We are hopeful that this workshop will consider these aspects as we discuss papers to be presented during the next couple of days. In looking over the agenda, we have some excellent papers on existing test methods and new test methods under development.

In closing, I would like to direct our attention to another requirement of the EPA under TSCA, we must not only consider the scientific value of the tests, but we must also consider the economic and resource limitations in applying proposed test methods to the testing of chemical substances. I would encourage discussion of these aspects along with the scientific utility of the test methods.

Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function
Neurobehavioral Toxicology, Vol. 1, Suppl. 1, p. 7. ANKHO International Inc., 1979.

Introduction and Overview

This Workshop was convened by the Environmental Protection Agency for the purpose of assessing the current capabilities of the discipline of Behavioral Toxicology for predicting neural and behavioral toxicity of environmental chemicals. Behavioral techniques can be employed to detect and establish dose-response relationships for toxicants for which the critical target is the nervous system. It has been pointed out in this meeting that behavioral tests may also detect effects upon systems other than the nervous system; i.e., establish indirect effects of toxicants, as well as make possible the identification of populations at greatest risk from a given toxicant.

Doctors Page, Tilson, Reiter, Gage and Seifter have emphasized the needs and priorities of regulatory agencies with regard to tests and methodologies for the detection and assessment of health effects. These requirements provide a challenge to behavioral scientists concerned with adapting the science of behavior to rigorous screening for behavioral toxicity. This challenge perhaps begins with the problem of definition of behavioral toxicity, which is obviously not an easy task. For convenience, a working definition probably should distinguish between acute, functional, reversible effects upon behavior, and chronic, structural damage to the nervous system which may or may not be associated with some degree of functional recovery from the primary deficit.

Other Aspects of the Challenge to Behaviorists Include

1. The need to maximize the amount of information obtainable from a behavioral test approach in order to evaluate effects upon overlapping functions, and to detect any degree of possible functional recovery. A corollary of this is the need for investigators to look for delayed effects upon behavior and for recovery from observed toxicities.
2. The need to refine estimates of the dose of toxicant to the nervous system. This may require an understanding of the metabolism of the initial exposure agent, identification of the specific neurotoxic chemical species, and establishment of the pharmacodynamic relationships involved.
3. The need to determine whether or not a dose-response relationship actually exists for all classes of neurotoxicants and what the limitations of specific behaviors are for the measurement of dose-response. There is some indication that acute or sub-chronic functional effects may plateau at low doses—perhaps due to saturation of receptors, or with the establishment of steady-state circulating and tissue reservoir levels. These considerations may be particularly important for substances administered by inhalation.
4. The need to evaluate and allow for differences between individual test animals in susceptibility to effects of a toxicant.
5. The need to consider whether enzyme induction or other effects of prior exposure to the test agent or to other compounds, treatments, etc., are affecting the outcome of the behavioral test.
6. The importance of testing for possible potentiation (or diminishment) of effects due to a given agent by the presence of other substances likely to be encountered in mixtures or adventitiously in the environment.
7. The sensitivity of the behavioral test and the relevance to the human situation may both be increased by the incorporation of a pharmacological challenge into the test.
8. The need to examine other possible treatment-behavior interactions including behavioral tolerance, sensitization-desensitization, hypersensitization, and compensation.

It is anticipated that the need of regulatory agencies for validated and comprehensive behavioral tests of nervous system function will provide clear-cut goals and objectives for behavioral scientists, and that the insights gained will result in greater coordination of research efforts—both with other behaviorists and with scientists in auxiliary disciplines such as biochemistry and pharmacology.

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Use of Discrimination Behavior for the Evaluation of Toxicants[1,2,3]

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GELLER, I., E. GAUSE, R. J. HARTMANN AND J. SEIFTER. *Use of discrimination behavior for the evaluation of toxicants.* NEUROBEHAV. TOXICOL. **1:** Suppl. 1, 9–13, 1979.—This study involved the application of discrimination behavior for the study of effects of environmental contaminants on the behavior of laboratory animals. Polybrominated biphenyl (PBB) was evaluated for effects on the acquisition and performance of a simple auditory discrimination by rats. Methyl ethyl ketone (MEK), methyl isobutyl ketone (MIBK) and carbon monoxide (CO) were evaluated for effects on a delayed match-to-sample discrimination task in the juvenile baboon. All of the contaminants slowed response times and increased extra responses. These findings suggest that discrimination behavior may be of value for the evaluation of environmental contaminants for effects on the central nervous system.

Polybrominated biphenyl   Ketones   Carbon monoxide   Environmental contaminants
Auditory discrimination   Delayed match-to-sample discrimination

THIS workshop focuses upon behavioral observations which may be useful for early detection of neurotoxicity attributable to environmental agents. Assessment of potential neurotoxicity becomes a formidable task because such a large number of functions are under nervous system control and these various functions may be inhibited differentially by any given neurotoxicant. In the detection of neuroactivity, discrimination tasks are useful because they lend themselves to the simultaneous measurement of a number of CNS mediated functions. For example, the delayed match-to-sample discrimination task can be said to include associative learning, visual reproduction (short-term memory), similarities or dissimilarities in stimuli, psychomotor function and response or reaction time. The potential value of such discrimination behavior in screening for neurotoxicity is indicated by the work of Hanninen who reported in this workshop [8] that humans exposed to toluene showed marked impairment of associative learning, visual reproduction, similarities and psychomotor function. This neurotoxicity of toluene appears to be quite specific since exposure to styrene, a structurally similar compound, did not affect results of the cognitive tests but did alter psychomotor function [8].

In the study of any type of toxicity, the relevance of rodent level observations for extrapolation to humans must be continually considered. Discrimination behavioral assays may be employed in both sub-human primates and rodents offering a direct inter-species comparison of the effects of a given agent as well as optimal relevance.

We have employed discrimination behavior to evaluate the toxicity of substances that are abused through inhalation and are also encountered as environmental or potential spacecraft contaminants. The absence of any clear cut information relative to the central nervous system effects of polybrominated biphenyl provided the impetus for a study on the effects of this toxicant on the acquisition and performance of a simple discrimination task by rats.

EXPERIMENT 1

METHOD

The animals were male Holtzman, Sprague-Dawley rats, approximately three months old at the start of the experiment.

A purified and analyzed sample of hexabrominated biphenyl, obtained from NIEHS, was prepared in lecithin-liposomes suspended in saline. It was administered orally at 1 mg/kg to 12 rats Monday through Friday of each week during a one-month period for a total of 20 doses of PBB. Twelve additional rats received 20 administrations of the control vehicle during the same one-month period.

For the discrimination task, hungry rats in Skinner boxes had to select the right or left lever as correct as a function of

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[1] Request for reprints should be addressed to Dr. I. Geller.

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[3] The authors are indebted to Murray Hamilton for tissue analysis of PBB's.

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the presence of a tone or clicker stimulus, respectively. The auditory stimuli occurred at random intervals on the average of once every two minutes (2-min VI). By making the correct choice, animals obtained milk rewards. Perfect discrimination was reflected in 100% correct responding to stimulus presentations with minimal or no responding in the absence of stimuli. Responses which occurred in the absence of stimuli reflected the general activity of the animal as well as a lack of efficiency.

Training on the discrimination task was as follows: all animals were gradually reduced to 80% of their original starting weights. They were then placed in the chambers for 1/2 hr, during which time the feeders were activated every 90 sec. On the following three days rats were placed in the chamber and given access to only the left lever. Pressing the lever would activate the clicker stimulus and produce a food reward. Animals remained in the chamber for 1/2 hr or until they made 100 responses. The right lever was then substituted for the left lever and animals received similar training in which a tone stimulus was paired with lever presses. After three days on this procedure, acquisition training for the discrimination task began. Tones or clicker stimuli occurred in a mixed order on the average of once every two minutes (2-min VI). Pressing the correct lever turned off the stimulus and activated the milk feeder. Pressing the incorrect lever simply turned off the stimulus. Response latencies for the entire session were cumulated on a running time meter.

RESULTS

PBB rats did not differ from controls with respect to accuracy on the discrimination task during the first four weeks of training. During Weeks 5–8 acquisition was more rapid for the control animals; however, the difference between PBB and control data was significant only on the eighth week of acquisition ($p<0.05$). From Week 9 onward, all rats performed at the 90% criterion level.

Throughout 24 weeks of discrimination training PBB rats were less efficient than controls in that they made many more extra responses. PBB rats also showed a trend toward longer response times throughout the experiment. Figure 1 shows these data for 18 weeks of training. Averaged weekly response time for PBB (solid lines) or control animals (broken lines) indicate PBB animals generally were slower to respond to either tone or clicker stimuli. The effect is most striking for response time measured on the right lever.

Ten months after the last PBB administration rats were sacrificed and analyzed for PBB levels by electron capture gas liquid chromatography. PBB was found in whole brain in concentrations ranging from 0.038 to 0.40 μg/g wet weight. PBB was also found in plasma in concentrations ranging from 0.135 to 0.372 μg/ml.

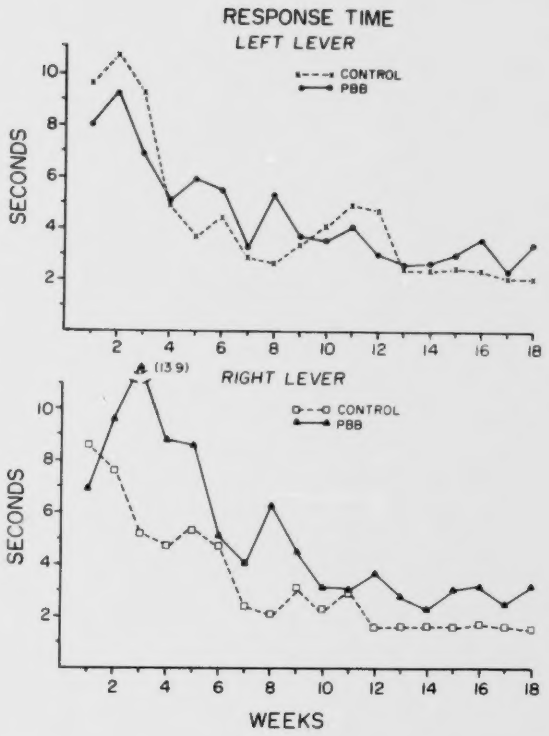

FIG. 1. Effect of polybrominated biphenyl on response time of the rat.

EXPERIMENT 2

METHOD

A match-to-sample discrimination task was used for the baboon and the toxicants were gases administered through inhalation in a flow-thru system.

The behavioral test chambers and gas exposure chambers have been previously described [7]. Two large stainless steel chambers equipped with a walk-in air lock were used to conduct the exposure. The chambers measured approximately nine feet high and nine feet in diameter. Juvenile male baboons approximately two years of age were housed in behavioral test chambers which were maintained in the large exposure chambers. The behavioral test chambers were designed so that an intelligence panel could be slipped down between the outside wall of the cage and the baboon. The intelligence panel was equipped with a row of three translucent discs which served as levers. Under the appropriate experimental conditions, pressing either side disc produced a banana pellet reward. Experimental sessions of two-hour duration were conducted on Monday through Friday of each week.

When the session timer was activated, a variable interval (VI) tape was set in motion. The tape programmed the occurrence of a stimulus on the center lever on the average of once every three minutes. The VI tape was inoperative during each trial which began with the illumination of one of the stimuli, the probe stimulus on the center lever. The stimulus was terminated after a 30-sec period or by a response on the lever. Termination of the stimulus activated a timer for a two-minute delay interval. At the end of the delay interval, stimuli appeared on both levers adjacent to the center lever. The correct matching stimulus was varied between these two levers in a mixed order. A response on the correct lever, where the stimulus matched the center lever stimulus, terminated the stimuli, activated the feeder and produced a banana pellet reward. Responses on the incorrect lever simply terminated the stimuli and again set the VI tape in motion.

A record was kept of the number of probe stimuli presented during each 15 min segment of a two-hr session, the numbers of correct matching responses on right and left levers and the number of incorrect responses. A record was also kept of any extra responses that may have occurred on the three levers when the stimuli were not activated or during the delay interval. The time it took the animal to press the lever after a stimulus was activated was also measured (response time). After the baboons were trained to 90–100% efficiency on the discrimination task, the exposure phase of the study was begun.

The animals were exposed to methyl ethyl ketone (MEK) or methyl isobutyl ketone (MIBK). Exposure was by means of the vapor saturation technique [3]. For the vapor saturation method, air is bubbled through a gas washing bottle containing the liquid to be vaporized. In passing through the liquid, the air becomes saturated with vapor which is then directed to the air intake ducts of the exposure chamber. Changing the flowrate with the fine metering valve or changing the temperature of the constant temperature bath allows one to produce a range of pollutant concentrations in the exposure chamber. The technique is simple and works well for substances that are liquids at room temperature. A Hewlett-Packard gas chromatograph, modified for automatic sampling was employed. This allowed for automatic sampling, quantitation and recording of pollutant concentrations in the exposure chamber.

The animals were also exposed to carbon monoxide (CO). The exposure atmospheres for the carbon monoxide studies were produced using compressed gas cylinders of CO obtained in 99.5% purity. The correct amount of carbon monoxide was introduced into the chamber by means of a calibrated flowmeter and a fine metering valve. Samples of chamber air were withdrawn with 1 ml gas tight syringe and analyzed on a gas chromatograph. The GC uses the principle of catalytic conversion to hydrogenate CO to methane which is detected with a conventional flame ionization detector. Samples were analyzed on the average of once every 10 min. The concentration was determined by a comparison of the detector response for a chamber air sample with the detector response for a series of standard samples. With these techniques, exposure chamber atmospheres were maintained within 10% of the desired value.

Animals were exposed to the ketones for 24 hr per day during a seven-day period. They were exposed to 100 ppm MEK, 50 ppm MIBK or to a combination of MEK or MIBK at the same concentrations. These concentrations are half the established threshold limit values [5]. While two animals in one of the chambers were being exposed to a contaminant atmosphere, the animals in the other chamber served as controls and were exposed to clean air during the same period. Thus, not only did other animals serve as controls, but each animal served as its own control in that exposure data could be compared with data obtained pre- and post-exposure time.

Animals were exposed to 25 ppm or 50 ppm CO for six hr per day during a one-week period while the two-hr behavioral test was conducted in the morning or afternoon. Thus, behavioral testing took place during the first two hr of CO exposure in the morning or in the afternoon after the animals had already been exposed for four hr.

RESULTS

For the ketones, performance on the match-to-sample task was not impaired under any of the three experimental conditions. However, response times or numbers of responses during the delay periods were affected by the gases in the four test animals.

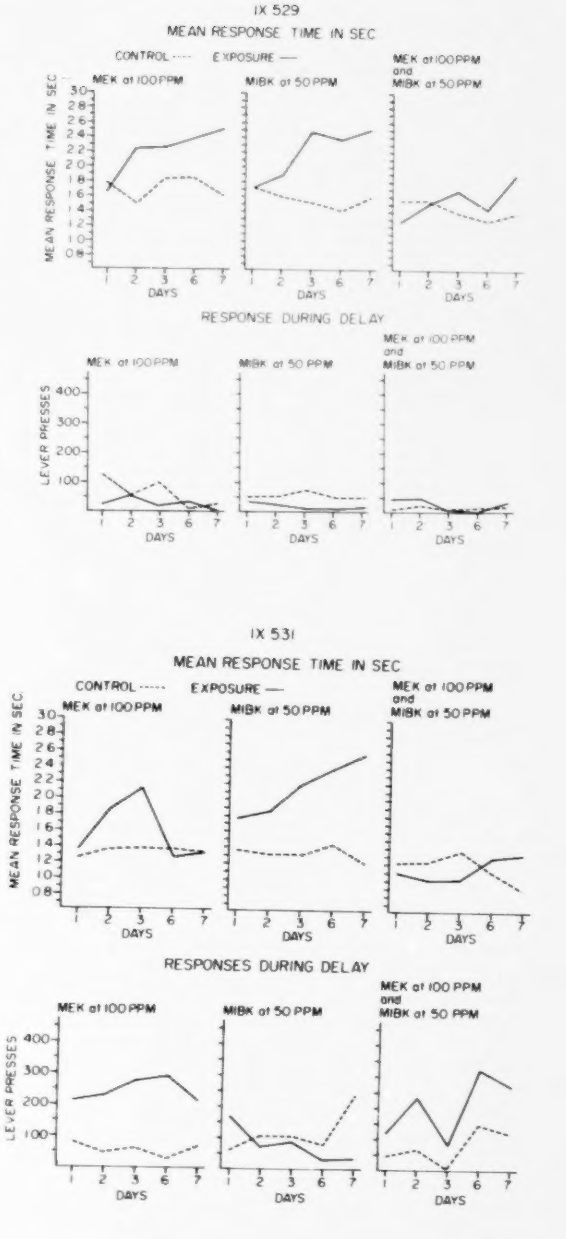

FIG. 2. Effect of seven-day exposures to 100 ppm MEK or 50 ppm MIBK, administered alone or in combination on match-to-sample behavior of baboons. Control data are represented by broken lines and exposure data by solid lines.

Figure 2 shows these effects in two baboons. The solid lines represent exposure data and the broken lines, control

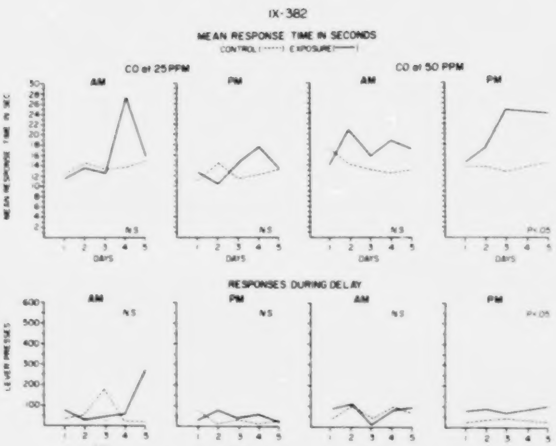

FIG. 3. Effects of six-hr daily exposure to carbon monoxide during a five-day period. Broken lines represent control data while solid lines represent exposure data.

data, obtained during a seven-day, pre-exposure period. For Baboon 529, mean response time increased above control levels under 100 ppm MEK during each of five behavioral sessions. The same was true for MIBK at 50 ppm. However, under a combination of MEK and MIBK at the same concentrations, the exposure data approximated that of the pre-exposure control. Mean response time for Baboon 531 increased gradually under 100 ppm MEK during the first three exposure days. On Days 6 and 7 the data were like that of controls. Mean response time under 50 ppm MIBK increased throughout the week of exposure. Again a combination of 100 ppm MEK and 50 ppm MIBK produced data similar to controls.

Responses during the delay intervals were like that of controls for Baboon 529, while for Baboon 531 there occurred a large increase under MEK, little or no effect under MIBK and an increase with the combined MEK, MIBK exposure.

For CO, a slight impairment of discrimination occurred at 50 ppm: animals exposed to this concentration of CO occasionally made a mistake.

Data typically obtained for response time latencies or responses during the delay are shown in Fig. 3: the broken lines in the figure represent control data averaged for each of five pre-exposure days and the solid lines represent data for five exposure days. At 25 ppm, CO produced a slowing of response time on Day 4 of the morning and afternoon exposures. These differences between exposed and control animals were not significant. Responses during the delay intervals did not change significantly under 25 ppm CO. The 50 ppm CO exposure produced a slowing of response time after Day 1 which persisted throughout the five-day period. This effect was significant for the afternoon animal who had already been exposed four hr each day when behavioral testing began. Responses during the delay interval increased significantly only for the afternoon animal during exposure to 50 ppm CO.

EXPERIMENTS 1 AND 2

DISCUSSION

Discrimination tasks have been used for the study of a number of psychoactive agents [6, 9, 10], and several rat studies have indicated that discrimination behavior may be of value for the study of certain central nervous system (CNS) active compounds [6,9]. It would appear that the application of discrimination behavior in a sub-human primate as well as in the rat should be a valuable technique of the relatively new field of behavioral toxicology. We have described here a simple discrimination task for the evaluation of toxicants in rats and a match-to-simple discrimination task for the evaluation of toxicants in young baboons. Application of these tasks to the study of effects of PBBs in rats, and to the study of effects of inhaled ketone vapors in baboons, is illustrated.

Rats treated with 1 mg/kg PBB were like controls with respect to accuracy on the discrimination tasks, however, extra responses and response latencies generally increased, thereby reducing the animal's efficiency.

Similarly, MEK or MIBK administered chronically at half the TLV over a seven-day period did not impair the baboons' ability to discriminate but did alter response latencies and extra responses during the delay intervals. The combinations of MEK and MIBK produced less effect on response latencies than did either one of the individual gases. Since the animals were esposed to the single gases prior to being exposed to the mixtures, it is possible that monooxygenases were induced in liver or extra-hepatic tissues that affected metabolism of the compounds on subsequent exposure, or that simultaneous inhalation of one compound affected the metabolism of the other compound. A loss of effect on response latency which occurred on the sixth and seventh day for two animals exposed to MEK alone might also be accounted for in terms of enzyme induction which increased metabolism of the inhaled gas.

The effects noted here with MEK and MIBK and the lessening of effect with a combination of the two vapors is of special interest. Both MEK and MIBK have been considered to be non-neurotoxic whereas methyl n-butyl ketone (MnBK), through the action of its metabolites, has been found to produce peripheral neuropathy, which is potentiated by simultaneous inhalation of MEK [1, 11, 12]. However, the potential CNS toxicity of MnBK has not been considered. The potentiation of the peripheral neurotoxicity of MnBK by MEK has also been associated with MEK-induced stimulation of microsomal enzyme activities: this effect is also manifested as a decrease in hexobarbital-induced sleep times [4]. However, these studies involved much higher vapor concentrations and longer exposure times than employed in the present studies.

The behavioral effects produced by MEK or MIBK at half the TLV concentrations indicate that the solvents are acting on the central nervous system. If the ketones rather than their metabolites are the neuroactive forms, enhanced metabolism might account for the observed loss of central nervous system effects in these studies. The consequences of human exposure to sub-TLV concentrations of these ketones should be evaluated.

Carbon monoxide increased extra responses during the delay interval while its greatest effect was to slow reaction time. Theodore *et al.* [13] reported a similar slowing of reaction times in monkeys exposed to almost twice the TLV of

CO (90 ppm). Our observation of minimal effects on the discrimination task itself are not in agreement with those of Beard and Wertheim [2] who reported disruption of an auditory discrimination task in humans exposed to CO at 50 ppm.

The findings with CO as well as with the ketones suggest that the match-to-sample discrimination task in the baboon provides a sensitive indicator of CNS effects of pollutants and should be evaluated further.

REFERENCES

1. Allen, N., J. R. Mendell, D. J. Billmaier, R. E. Fontaine and J. O'Neill. Toxic polyneuropathy due to methyl n-butyl ketone. *Arch Neurol.* **32:** 209–222, 1975.
2. Beard, R. R. and G. A. Wertheim. Behavioral impairment associated with small doses of carbon monoxide. *Am. J. publ. Hlth* **57:** 2012–2022, 1967.
3. Cotabish, H. N., P. W. McConnaughey and H. C. Messer. Making known concentrations for instrument calibration. *Am. ind. Hyg. Ass. J.* **22:** 392–402, 1961.
4. Couri, D., L. B. Hetland, M. S. Abdel-Rahman and H. Weiss. The influence of inhaled ketone solvent vapors on hepatic microsomal biotransformation activities. *Toxicol. appl. Pharmac.* **41:** 285–289, 1977.
5. Documentation of Threshold Limit Values: *Am. Conf. Gov. ind. Hyg.* 3rd Edition, 1971.
6. Geller, I., R. J. Hartmann and K. Blum. Effects of nicotine, nicotine monomethiodide, lobeline, chlordiazepoxide and caffeine on a discrimination task in laboratory rats. *Psychopharmacologia* **20:** 355–365, 1971.
7. Geller, I., R. L. Martinez, R. J. Hartmann and H. L. Kaplan. Effects of ketones on a match-to-sample task in the baboon. *Proc. West. Pharmac. Soc.* **21:** 439–442, 1978.
8. Hanninen, H. Psychological test methods sensitivity to long-term chemical exposure at work. *Neurobehav. Toxic.* (IN PRINT).
9. Hughes, F. W. and E. Kopmann. Influence of pentobarbital, hydroxyzine, chlorpromazine, reserpine and meprobamate on choice discrimination behavior in the rat. *Arch. Int. Pharmac. Ther.* **CXXVI:** 158–170, 1960.
10. Poling, A., M. A. Simmons and J. B. Appel. Morphine and shock detection: effects on shock intensity. *Commun. Psychopharmac.* **2:** 333–336, 1978.
11. Saida, K., J. R. Mendell and H. S. Weiss. Peripheral nerve changes induced by methyl n-butyl ketone and potentiation by methyl ethyl ketone. *J. Neuropathol. Exp. Neurol.* **XXXV:** 207–225, 1976.
12. Spencer, P. S. and H. H. Schaumburg. Feline nervous system response to chronic intoxication with commercial grades of methyl n-butyl ketone, methyl isobutyl ketone and methyl ethyl ketone. *Toxic. appl. Pharmac.* **37:** 301–311, 1976.
13. Theodore, J., R. D. O'Donnell and K. C. Back. Toxicological evaluation of carbon monoxide in humans and other mammalian species. *J. occup. Med.* **13**(5): 242–255, 1971.

Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function
Neurobehavioral Toxicology, Vol. 1, Suppl. 1, pp. 15–22. ANKHO International Inc., 1979.

Effects of Toxicants on Visual Systems[1]

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MERIGAN, W. H. *Effects of toxicants on visual systems.* NEUROBEHAV. TOXICOL. **1:** Suppl. 1, 15–22, 1979.—The analysis of visual toxicity is complicated by the heterogeneity of visual capacities in different regions of the visual field. Since various toxicants may impair different functions allied to localized portions of the visual field, it is important to explore the relationship of field defects to residual visual abilities. We have begun this exploration by studying methylmercury poisoning in macaque monkeys. Extended exposure to this toxicant produces a marked concentric constriction of visual fields, a result similar to that found in human victims. In addition, visual sensitivity is greatly reduced on those tests in which the periphery of the visual field is more sensitive than the center. Our findings suggest simple but reliable clinical tests for screening suspected victims of substances impairing peripheral vision.

Visual fields   Visual thresholds   Methylmercury   Neurotoxicity

VISION, which in humans may be the most important of the exteroceptive senses, is extremely sensitive to toxic insult. Many toxicants, including methanol, carbon disulfide, some arsenicals, methylmercury and pesticides, act directly on the visual system. Numerous other toxic substances, such as lead and carbon monoxide may cause visual impairment as a result of less specific damage to the central nervous system.

While visual toxicity can be assessed with the techniques of physiology and pathology, the testing of visual capacities remains the most direct way to detect impaired vision. Visual testing is superior to other techniques in that it is non-invasive, it does not provoke great anxiety in patients, and it is suitable for field testing of industrial workers or suspected victims of environmental pollution.

Unfortunately, the measurement of vision is complicated by the inhomogeneity of visual capacities in different portions of the visual field. For example, the portion of the visual field located at the center of gaze, the fovea, has considerably greater visual acuity than other regions of the visual field (Fig. 1). Thus, visual impairment confined to the foveal region would cause a much more severe loss of acuity than damage in extrafoveal regions.

These considerations are of particular importance in visual toxicity because of the marked specificity of many toxicants for certain portions of the visual field. Figure 2 shows examples of substances which can cause localized damage of the visual field. The polar plots represent the central 30° of the visual field of each eye with the origin located at the fovea. The optic disc or blind spot, which is not shown, is located between about 13 and 17° eccentricity on the temporal (outer) side of each visual field. The blackened area of the plots represents a reduction of visual sensitivity (scotoma) but not necessarily a complete loss of vision.

Consumption of methanol (methyl alcohol) produces a marked edema of the optic disc and typically results in a central or circumcentral loss of vision [9,17]. Prolonged malnutrition (Fig. 2) frequently causes optic atrophy and small bilateral central scotomas [8]. Harrington [8] has pointed out that the common finding of central field defects in the so-called "tobacco and alcohol amblyopias" may also be due to a Vitamin B12 deficiency in some alcoholics and heavy smokers. Lead, carbon disulfide, thallium, and several drugs can also induce scotomas in the center of the visual field [7, 8, 18].

A loss of peripheral vision is characteristic of exposure to other substances [7,8]. Figure 2 shows an almost complete loss of vision following idiopathic quinine poisoning but with some residual central vision. Salicylate poisoning is quite rare but results in a similar field constriction. Peripheral field loss can also be caused by chloroquine, pentavalent arsenicals, carbon monoxide and some drugs. Both central and peripheral impairment of the visual field may follow exposure to nitrobenzol, dinitrobenzene and methylbromide. An example of such a loss is shown in Fig. 2 for nitrobenzol.

The locus of pathological changes in the disorders described above is most frequently the retina or optic nerve. Central visual defects could be due to selective toxicity of cone photoreceptors which predominate near the fovea, but the usual basis is damage to the papillo-macular bundle of nerve fibers which serves the foveal region [8]. Peripheral losses most often result from damage to ganglion cells or the pigment epithelium [7]. However, since the visual field remains topographically segregated throughout the more central portions of the visual system (e.g., in the lateral geniculate, striate cortex and pre-striate areas) visual field defects could be produced by lesions in any of these structures. The

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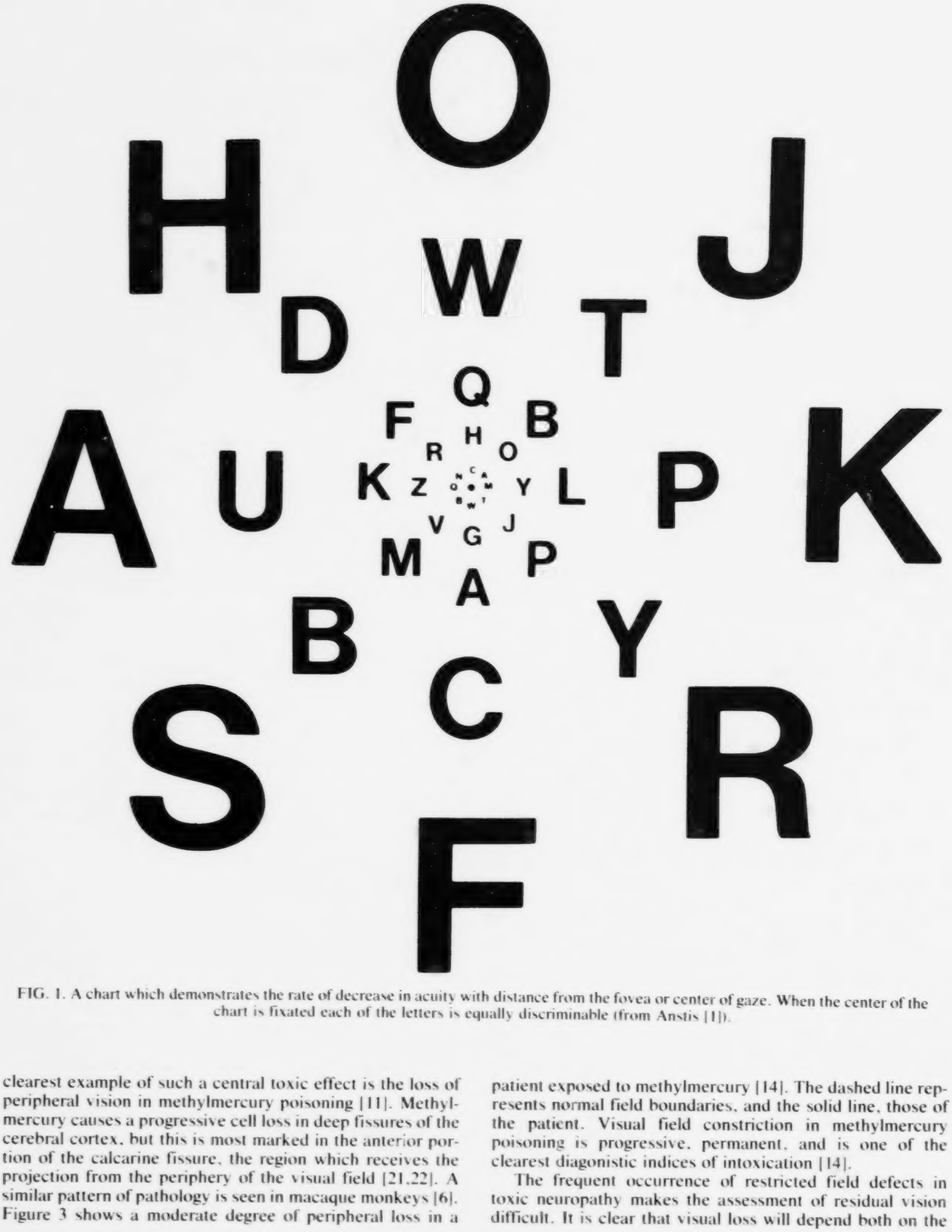

FIG. 1. A chart which demonstrates the rate of decrease in acuity with distance from the fovea or center of gaze. When the center of the chart is fixated each of the letters is equally discriminable (from Anstis [1]).

clearest example of such a central toxic effect is the loss of peripheral vision in methylmercury poisoning [11]. Methylmercury causes a progressive cell loss in deep fissures of the cerebral cortex, but this is most marked in the anterior portion of the calcarine fissure, the region which receives the projection from the periphery of the visual field [21,22]. A similar pattern of pathology is seen in macaque monkeys [6]. Figure 3 shows a moderate degree of peripheral loss in a patient exposed to methylmercury [14]. The dashed line represents normal field boundaries, and the solid line, those of the patient. Visual field constriction in methylmercury poisoning is progressive, permanent, and is one of the clearest diagonistic indices of intoxication [14].

The frequent occurrence of restricted field defects in toxic neuropathy makes the assessment of residual vision difficult. It is clear that visual loss will depend both on the

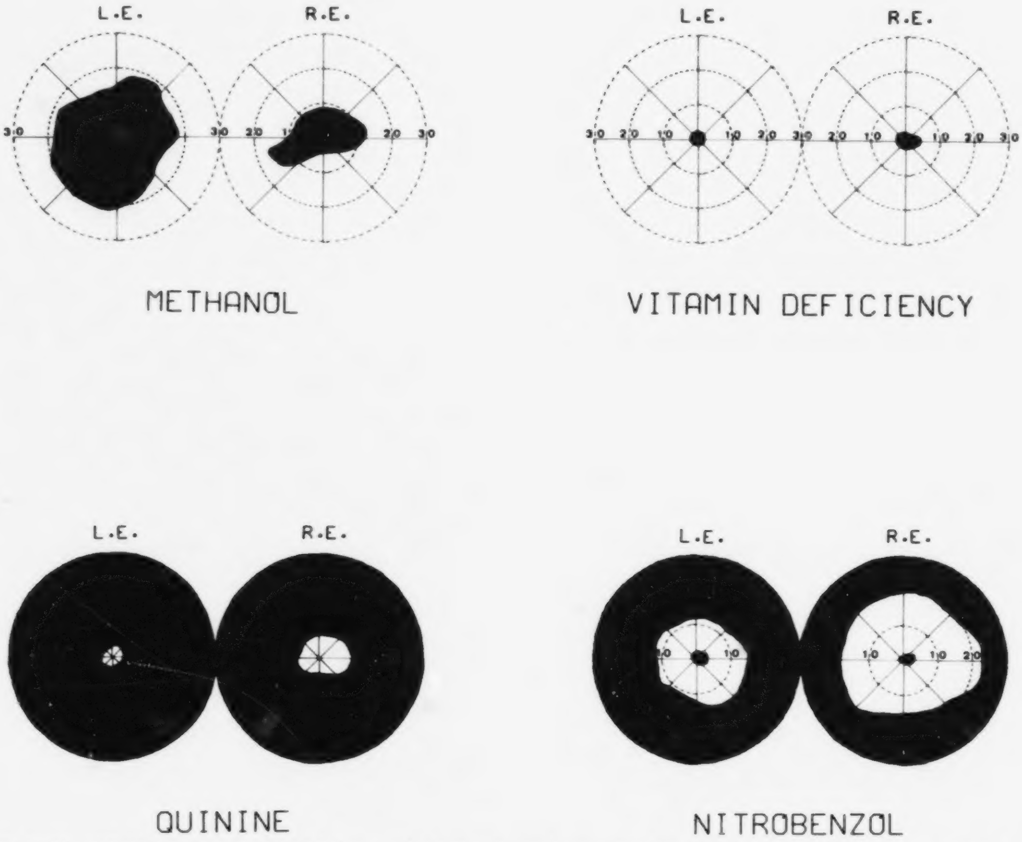
L.E.
R.E.
METHANOL
L.E.
R.E.
VITAMIN DEFICIENCY
L.E.
R.E.
QUININE
L.E.
R.E.
NITROBENZOL

FIG. 2. Visual field plots showing field defects typical of toxic syndromes. Each polar plot represents the central 30° of vision for the right (R.E.) and left (L.E.) eyes. Blackened areas show regions of reduced visual sensitivity. (Adapted from Harrington [8]).

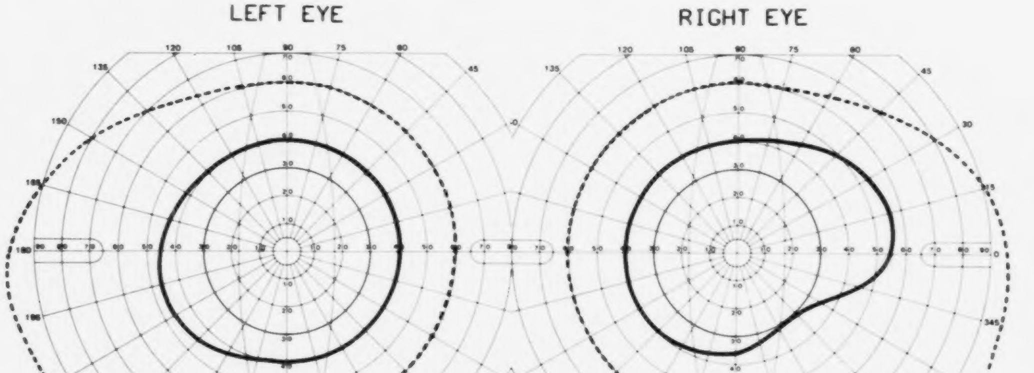

FIG. 3. Visual field charts showing a moderate concentric constriction of visual fields in methylmercury poisoning [14]. The dashed lines indicate normal visual fields and the solid line the field boundaries of a victim of methylmercury.

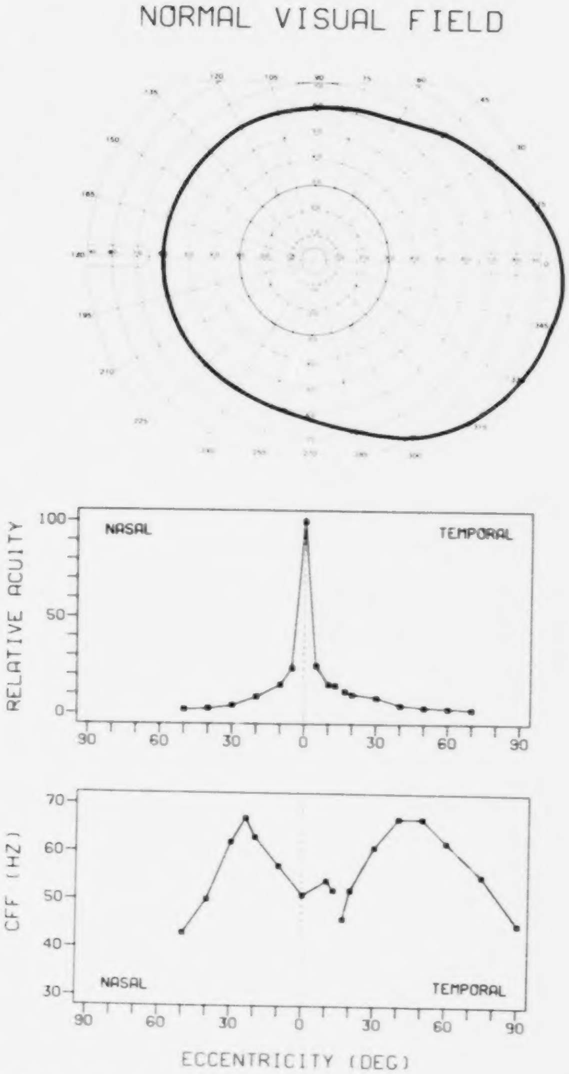

FIG. 4. The sensitivity profile across the visual field for two types of visual thresholds. The upper plot shows the normal boundary of the visual field which reaches its greatest eccentricity in the inferior temporal region. The middle graph demonstrates the abrupt decrease in visual acuity with eccentricity from the fovea (from Fick [5]). The bottom graph shows how flicker fusion frequency (CFF) reaches a maximum at 30 to 50° of eccentricity when the flickering stimulus subtends 3° visual angle [12]. The interrupted portion of the acuity and CFF functions represents the location of the blind spot.

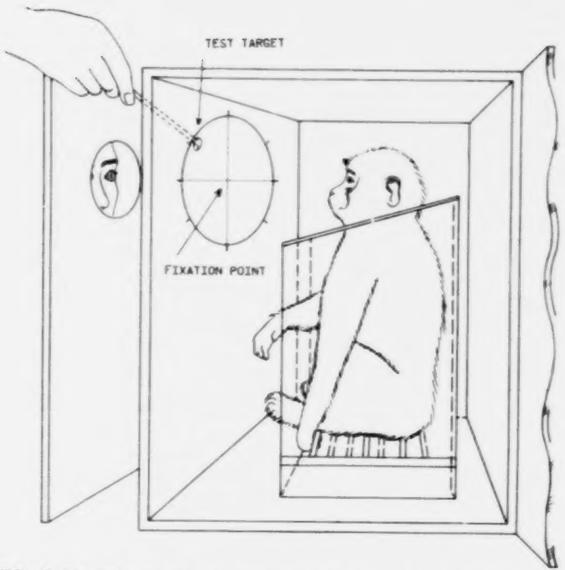

FIG. 5. Technique used to measure the boundaries of the visual fields of monkeys.

location of the defect and on the visual capacities in that location. The profile of sensitivity throughout the visual field for various types of stimuli has been mapped using the technique of static perimetry [2]. In general, visual sensitivity is similar in those regions equidistant from the fovea. However, sensitivity can either increase or decrease with eccentricity from the fovea depending on the stimulus. Figure 4 shows two different sensitivity profiles measured in a horizontal plane across the visual field passing through the fovea. The polar plot shows the normal boundary of the visual field; extending to greater eccentricity in the temporal than nasal direction. The middle graph shows the abrupt decrease of visual acuity with increasing distance from the fovea [5]. Sensitivity to motion [23] and color [20] also decreases outside the fovea. On the other hand, the bottom graph in Fig. 4B shows that the maximum detectable rate of flicker (critical fusion frequency or CFF) increases with eccentricity to peak at about 30 to 50°. This type of profile is only found for larger flickering stimuli; for stimuli smaller than 0.5° of visual angle, CFF is greatest in the fovea [12]. Low luminance or night vision also shows greatest sensitivity outside the foveal region [2].

A comparison of Figs. 2 and 3 with Fig. 4 suggests that victims of methanol and Vitamin B12 deficiency will suffer a loss of visual acuity, while those poisoned with quinine or methylmercury should have difficulty with large flickering targets. This requires, of course, that the victim is using the most sensitive part of the visual field for the task at hand. This assumption may be safer for central vision (we don't notice our poor peripheral acuity because we aim our fovea at objects of interest) than for peripheral vision. In any case, it is clear that stimuli used to detect visual loss should be carefully chosen to reflect the different properties of central and peripheral vision. Such stimuli offer some advantages as diagnostic procedures over static perimetry in that less equipment, testing time, patient cooperation and examiner skills are required. These qualities make such tests useful for large scale screening of industrial workers of field studies of populations suspected of being at risk. In addition, these visual tests could be used to develop animal models for studies of mechanisms of visual toxicity.

The work I will describe below was aimed at the development and validation of such a model of primate intoxication with methylmercury. We measured flicker thresholds in

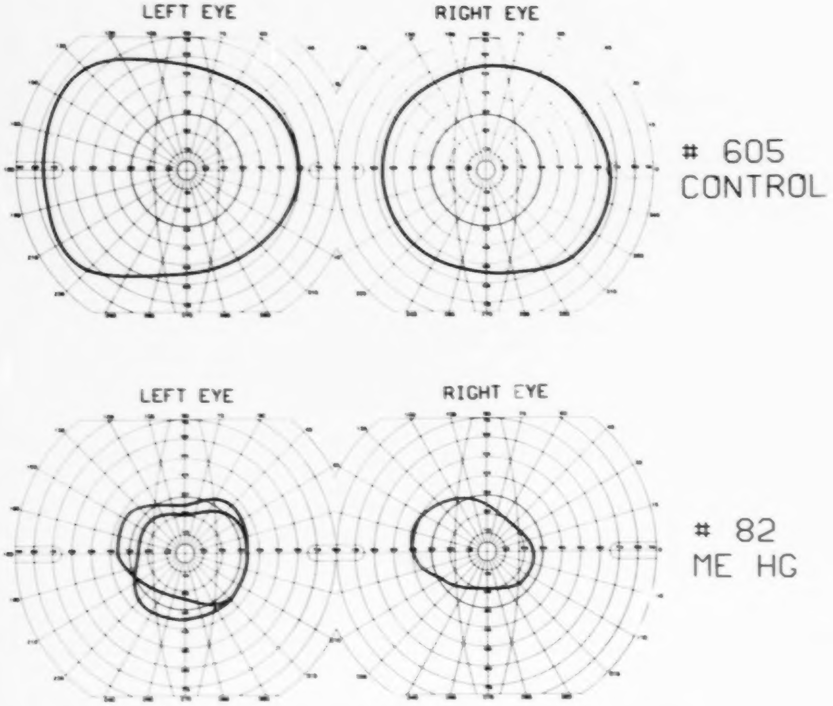

FIG. 6. Visual field boundaries of a control and a methylmercury poisoned monkey.

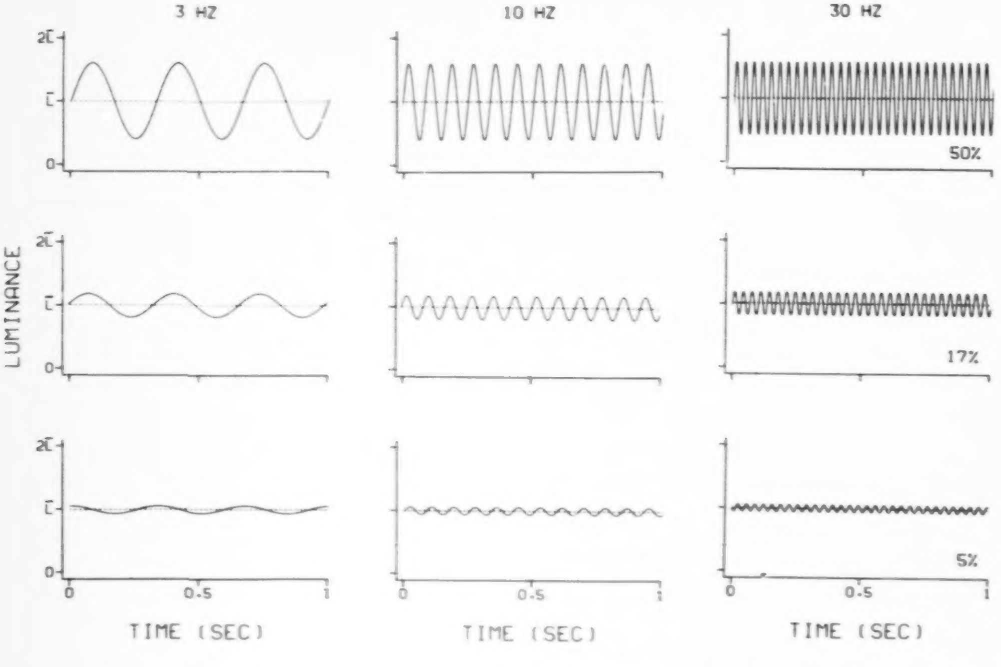

FIG. 7. Representation of the temporal luminance profile of representative flickering test stimuli. Each plot shows that the target luminance was modulated in time around the mean luminance (L̄). Three modulation depths are shown for each of three frequencies of flicker

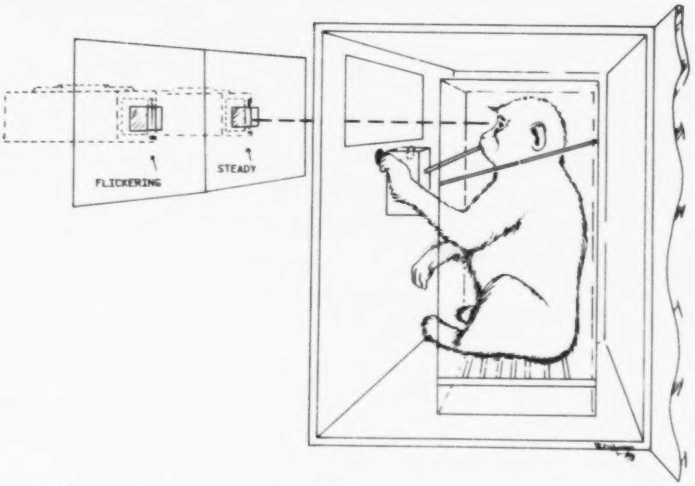

FIG. 8. Technique used to measure flicker sensitivity of monkeys. The monkey is responding correctly on the left pushbutton which corresponds to the location of the flickering display. Juice reward is delivered through a stainless steel drinking spout.

a monkey which had received sufficient methylmercury to produce a moderate constriction of visual fields. We were especially interested in those measures of flicker sensitivity which are most dependent on the periphery of the visual field. The methodological details are more fully described elsewhere [16].

Animals

Results will be reported for two macaque monkeys, one of which served as a control (No. 605) while the other (No. 82) was treated with methylmercury. The exposure history of Monkey 82 has been reported [16]. Monkey 82 showed a marked myopia but was corrected during testing with corneal contact lenses.

Perimetry

The technique used for visual field measures is shown schematically in Fig. 5. The monkey sat in an acrylic test chair facing the circular aperture which was used to determine the limits of the visual field. The visual subtense of this aperture could be changed in increments of 10° from 10 to 70° by moving the restraining chair or by inserting panels with smaller openings. The tester attracted the monkey's gaze by placing a small piece of fruit or a marshmallow at the fixation point. When the monkey fixated this central target, a 1 cm white marshmallow was moved inside the aperture at one of the eight test points located at 45° intervals. If the monkey glanced toward the test marshmallow, this detection was marked at the appropriate location on the visual field chart. A range of eccentricities was tested at each location to determine the limits of the field. All testing was monocular and the non-tested eye was occluded with an opaque contact lens. To prevent habituation, the pieces of food used as fixation and test stimuli were frequently extended to allow the monkey to eat them.

The visual field plots of the control (No. 605) and poisoned (No. 82) monkeys are shown in Fig. 6. The results for Monkey 605 are typical of normal monkeys tested in our laboratory. The visual field extends to approximately 50° in the nasal and superior directions and to at least 65° in the temporal direction. Where the field extended beyond the 70° limit of our perimeter, it was marked as 75°. Targets presented at more than 50° eccentricity in the inferior direction were partially blocked by the acrylic chair.

The visual fields of Monkey 82 show a marked constriction compared to those of the control monkey. The two plots for the left eye of this monkey represent determinations separated by several days. All measures indicated a concentric constriction of visual fields to about 35 to 40° of eccentricity. Such a pronounced field constriction should be correlated with a decreased sensitivity to stimuli which depend primarily on the periphery of the visual field for detection. Thus, we tested Monkey 82 and the control monkey to explore the feasibility of using such stimuli to detect visual loss in methylmercury poisoning.

Flicker Sensitivity

The measures of flicker sensitivity will be described by analogy to the similar but more familiar quantification of audition by the audiogram. The stimuli we use are shown schematically in Fig. 7. While the audiologist uses pure (sinusoidal) tones, we use sinusoidal flicker of a luminous target. The audiogram measures audibility of tones of 50 to 10,000 Hz while we test the visibility of flicker rates of 2 to about 70 Hz. Figure 7 illustrates flicker test stimuli of 3, 10, and 30 Hz. The measure of auditory sensitivity is threshold loudness (sound pressure amplitude) while flicker sensitivity threshold is expressed as relative luminance modulation depth. Modulation depth is defined as $\frac{\text{LMAX - LMIN}}{\text{LMAX + LMIN}}$ where LMAX is the maximum and LMIN the minimum luminance of the flickering stimulus. Flicker modulation depths of 50, 17, and 5% are shown in Fig. 7. A final parameter of our measures is the mean or adapting luminance of our stimuli ($\bar{L}$ in Fig. 7), since light or dark adaptation changes visual sensitivity much as adapting sound level changes auditory sensitivity. Flicker sensitivity was exam-

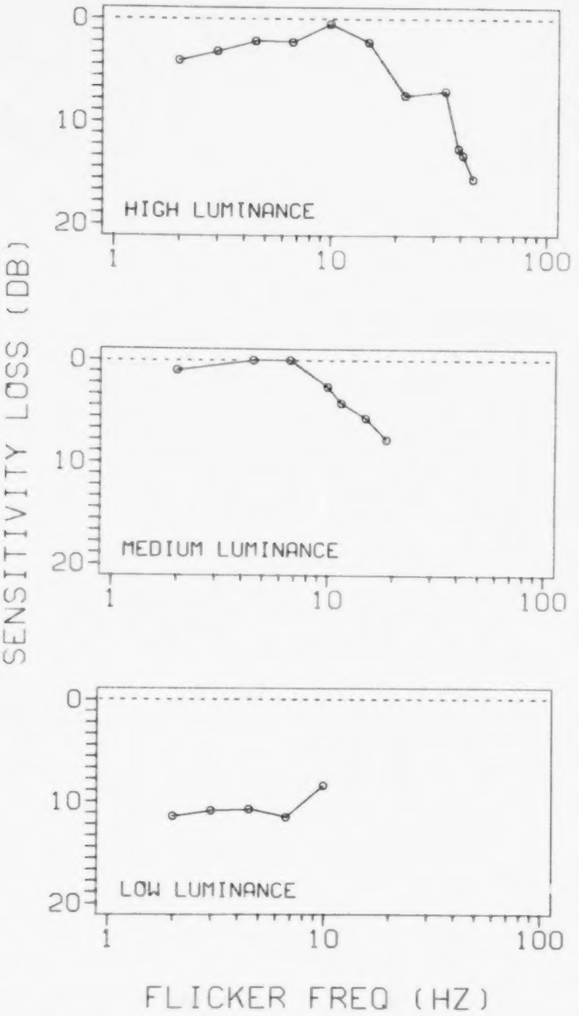

FIG. 9. Flicker visuogram of the methylmercury poisoned monkey. The dashed line represents the modulation sensitivity of the control monkey. Distance below the dashed line indicates the degree of sensitivity loss. High luminance = 5 ft L, medium luminance = 0.05 ft L, low luminance = 0.0005 ft L.

ined at 3 luminances: one at which cone photoreceptors are dominant (high luminance), one at which only rod photoreceptors are active (low luminance), and finally at an intermediate level (medium luminance) which is slightly above the point of transition from rod to cone vision. Detection of the low luminance stimuli is strongly dependent on the periphery of the visual field [10]. In addition, at the medium and high luminances used in this study, the detection of high rates of flicker depends on peripheral vision [12] (see Fig. 4 above).

The actual arrangement of the test apparatus is illustrated in Fig. 8. The seated monkey faced two display oscilloscopes at a distance of 1 m. The face of each display was evenly illuminated and subtended 4.6×5.7° of visual angle. On each trial the entire illuminated portion of one of the two displays was flickered. The monkey indicated the flickering display by pressing the corresponding pushbutton on the test panel. Correct choices were rewarded with a small amount of fruit juice.

The frequency and modulation depth of flicker were set remotely by the on-line computer which controlled the experiments. The mean luminance of the stimuli was varied by placing neutral density filters over the face of each display. In each session, sensitivity to a single flicker frequency at one luminance level was tested. Modulation thresholds were measured by presenting flicker at 5 modulation depths, chosen to bracket the threshold value, in random order. The threshold was defined by interpolation on the resulting psychometric function as the modulation depth at which performance fell to 75% correct.

The results in Fig. 9 show the sensitivity loss of the poisoned monkey relative to the control monkey over the range of flicker frequencies tested. The most striking finding at high and medium luminance was a great loss of sensitivity to high frequencies of flicker. The maximum resolvable flicker frequency (CFF) at high luminance was only 45 Hz in the poisoned monkey compared to 70 Hz in the control. The role of visual field constriction in this reduced sensitivity to high frequency flicker can easily be seen by comparing Figs. 4 and 6. Figure 6 shows that the visual fields of Monkey 82 reach only to 35 or 40° of eccentricity. In addition, detailed studies of human methylmercury victims [14] indicate that visual impairment extends well inside the limits of the visual field. Thus, Monkey 82 has suffered a severe impairment of the very regions of the visual field which can resolve the highest rate of flicker (see Fig. 4).

The slight loss of sensitivity to low rates of flicker shown in the upper part of Fig. 9 is somewhat more puzzling because the visibility of such stimuli is not thought to depend on peripheral vision. This loss could be due to reduced acuity, since the perception of low flicker frequencies is greatly enhanced by the presence of sharply focussed edges [13]. However, since Monkey 82 shows only a slight loss of visual acuity (20% below the mean of normal monkeys we have tested) it is unlikely that the low frequency flicker loss is due to poor pattern vision. Further studies will be required to determine if low frequency impairment is a consistent sign of methylmercury intoxication.

The bottom panel in Fig. 9 shows a profound loss of sensitivity for all frequencies of low luminance flicker. This result is consistent with the observation [10,15] that the periphery is more responsive to flicker at extremely low luminances than is the center of the visual field. Thus, low luminance as well as high frequency flicker sensitivity is greatly reduced in methylmercury poisoning and this is correlated with a constriction of visual fields.

Stimuli which are detected most easily with central vision should prove poor indicators of methylmercury induced visual loss. Indeed, methylmercury victims in Iraq showed no impairment of visual acuity [19]. A particularly dramatic illustration of residual central vision is the report of Iwata [14] that CFF was unimpaired in a large population of methylmercury victims. At first glance this result appears inconsistent with our findings. However, the results of Hylkema [12] show that this apparent inconsistency is due to the size of the test stimulus. For small flickering stimuli such as those used by Iwata, CFF is maximal at the fovea: thus, there is no impairment of methylmercury poisoning. However, for large targets, such as those used to test Monkey 82, CFF increases with eccentricity (Fig. 4). Thus, CFF could be used for detection of either central or peripheral field loss depending on the size of the target.

The present findings as well as those of previous investigations can be used to devise clinical tests for visual loss in methylmercury poisoning. Measurements of the boundaries or the sensitivity profiles across the visual field provide detailed descriptions of early visual loss [14]. The major drawback of these techniques is that they require a cooperative patient and a skilled examiner. The measurement of low luminance vision [3,4] gives an early index of intoxication which is applicable to studies of non-human primates. Iwata [14] has achieved even greater sensitivity in the detection of toxicity by blocking the central 40° of the visual field and measuring low luminance vision with evoked responses. Unfortunately, these techniques require a lengthy dark adaptation period and a subject who is cooperative in the dark. On the other hand, the data from Monkey 82 suggest that the measurement of CFF with a large flickering target may provide the most convenient early index of methylmercury intoxication. This test could be used for the rapid screening of populations potentially intoxicated with methylmercury. Studies are underway in our laboratory to determine if this simple measure is as early and reliable an index of poisoning as the more difficult examinations described above.

REFERENCES

1. Anstis, S. M. A chart demonstrating variations in acuity with retinal position. *Vision Res.* **14:** 589–592, 1974.
2. Aulhorn, E. and H. Harms. Visual perimetry. In: *Handbook of Sensory Physiology, Vol. VII (4) Visual psychophysics*, edited by D. Jameson and L. Hurvich. Berlin: Springer, 1973.
3. Berlin, M., C. Grant, J. Hellberg, J. Hellstrom and A. Shutz. Neurotoxicity of methylmercury in squirrel monkeys. *Archs envir. Hlth.* **30:** 340–348, 1975.
4. Evans, H. L. Early methylmercury signs revealed in visual tests. In: *Proc. Intern. Conf. on Heavy Metals in the Environment*, Vol. 3, edited by T. C. Hutchinson. Toronto: University of Toronto, 1977, pp. 241–256.
5. Fick, A. E. Ueber Stabchensehscharfe und Zapfensehscharfe. *Albrecht von Grafe's Archiv fur Ophthalmologie* **45:** 336–356, 1898.
6. Garman, R., B. Weiss and H. Evans. Alkylmercurial encephalopathy in the monkey (*Saimiri sciureus* and *Macaca arctoides*). *Acta Neuropath.* **32:** 61–74, 1975.
7. Grant, W. M. *Toxicology of the Eye.* Springfield, Ill.: Charles C. Thomas, 1974.
8. Harrington, D. O. *The Visual Fields.* St. Louis: C. V. Mosby Company, 1976.
9. Hayreh, M. S., S. S. Hayreh, G. Baumbach, P. Cancilla, G. Martin-Amat, T. R. Tephly, K. E. McMartin and A. B. Makar. Methanol poisoning: III. Ocular toxicity. *Archs Ophthalmol.* **95:** 1851–1858, 1977.
10. Hecht, S. and C. D. Verrijp. Intermittent stimulation by light. III. The relation between intensity and critical fusion frequency for different retinal locations. *J. gen. Physiol.* **17:** 251–265, 1933a.
11. Hunter, D., R. R. Bomford and D. S. Russell. Poisoning by methylmercury compounds. *Q. J. Med.* **9:** 193–213, 1940.
12. Hylkema, B. S. Examination of the visual field by determining the fusion frequency. *Acta opthal. (Kbh.)* **20:** 181–193, 1942.
13. Kelly, D. H. Flickering patterns and lateral inhibition. *J. Optical Soc. Am.* **59:** 1361–1370, 1969.
14. Iwata, K. Neuroophthalmological findings and their transition in "Minimata Disease" organic mercury poisoning in Agano area of Niigata perfecture. *Acta soc. ophthal. japonica.* **77:** 1788–1834, 1974.
15. Lloyd, V. V. A comparison of critical fusion frequency for different areas in the fovea and the periphery. *Am. J. Psychol.* **65:** 346–357, 1957.
16. Merigan, W. H. Visual fields and flicker thresholds in methylmercury poisoned monkeys. In: *Neurotoxicity of the Visual System*, edited by W. H. Merigan and B. Weiss. New York: Raven Press, in press.
17. Potts, A. M. The visual toxicity of methanol: VI. The clinical aspects of experimental methanol poisoning treated with base. *Am. J. Opthalmol.* **39:** 86–92, 1955.
18. Potts, A. M. and L. M. Gonasun. Toxicology of the eye. In: *Toxicology: The Basic Science of Poisons*, edited by J. Casarett and J. Doull. New York: McMillan, 1975.
19. Sabelaish, S. and G. Hilmi. Ocular manifestations of mercury poisoning. *Bull WHO Suppl.* **53:** 83–86, 1976.
20. Sperling, H. G. and Y. Hsia. Some comparisons among spectral sensitivity data obtained in different retinal locations and with two sizes of foveal stimulus. *J. Opt. Soc. Am.* **47:** 707–713, 1957.
21. Takeuchi, T. Neuropathology of Minimata disease in Kumamoto: especially at the chronic stage. In: *Neurotoxicology*, edited by L. Roizin, H. Shiraki and N. Grcevic. New York: Raven Press, 1977.
22. Takeuchi, T., N. Morikawa, H. Matsumoto and Y. Shiraishi. A pathological study of Minimata disease in Japan. *Acta Neuropathol. (Berl)* **2:** 40–57, 1962.
23. Tynan, P. D. Motion processing in peripheral vision: reaction time, contrast threshold and perceived velocity. Unpublished dissertation, Northwestern University, 1979.

Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function
Neurobehavioral Toxicology, Vol. 1, Suppl. 1, pp. 23–31. ANKHO International Inc., 1979.

Effects of Toxicants on the Somatosensory System

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MAURISSEN, J. P. J. *Effects of toxicants on the somatosensory system.* NEUROBEHAV. TOXICOL. **1:** Suppl 1, 23–31, 1979.—A computerized system for an objective and accurate study of vibration sensitivity has been designed. Sensitivity can be assessed in human as well as nonhuman primates. Its usefulness in the study of peripheral nerve disorders induced by chemical exposure is emphasized.

Vibration sensitivity Sensory disturbances Neurotoxicants

NUMEROUS chemicals (drugs and toxic chemicals) are known to produce peripheral sensory neuropathy. Morphological, electrophysiological and clinical studies have attempted to define the exact nature of these neurotoxic effects. Morphological studies have investigated the anatomical structure and ultrastructure of peripheral nerves. Electrophysiological studies have emphasized the importance of a functional approach to these disorders. However, electrophysiological measures (nerve conduction velocity, somatosensory evoked potentials) do not always reflect neurological status. Clinical studies have aimed at detecting the presence of frank disorders but do not have the refinements necessary to detect early signs of peripheral nerve damage, or to follow sensory recovery after exposure to a neurotoxicant.

Still another approach to these problems can be taken. Psychophysics is the scientific and objective study of the relations between stimuli and resulting sensations. Operant and Pavlovian conditioning offer the possibility of studying sensory processes in animals psychophysically.

This paper will briefly review the methods used to study the effects of neurotoxic chemicals on somesthetic sensitivity and will describe a technique for the study of vibration sensitivity in human and nonhuman primates.

METHODS FOR STUDY OF SOMESTHETIC SENSITIVITY

Clinical examination is essentially the sole source of information about the effects of chemicals on sensory functions. However, uncontrolled factors make it a crude indicator of sensory status.

Scope and Limitations of Neurological Examination

Sensory testing is an important part of the neurological examination. Two-point discrimination is generally tested with a pair of calipers, pain sensitivity with the "neurologic pin," thermal sensitivity with tubes containing hot or ice-cold water, light touch with a cotton wisp, and vibration sensitivity with a tuning fork. These techniques are designed to detect frank sensory abnormalities. They are suitable neither for detecting a partial sensory loss, nor for following sensory recovery after peripheral nerve insult. The stimuli used are also hardly reproducible. There is no standard way to apply them and in no case is the velocity of impact on the skin controlled. Furthermore, evaluation of the subject's performance is highly subjective. For example, the examiner strikes a tuning fork with an unknown and irreproducible force, applies its base on a bony eminence with an unknown pressure, and asks the subject to report when vibration ceases to be felt. At that time, the tuning fork typically is applied to a similar part of the examiner's body. If the examiner still perceives vibration, sensitivity of the subject is diagnosed as impaired. Even if the sensitivity of the examiner stays constant over time, it is not possible to compare data obtained by different examiners.

Necessity for Investigative Techniques

Several pieces of equipment have been designed for a more quantitative and objective evaluation of somesthetic sensitivity. Unfortunately they are not being introduced in clinical practice very eagerly. Temperature sensitivity can be assessed with simple, inexpensive and commercially available thermal stimulators [33] or with more complex thermoelectric units whose temperature can be very accurately controlled [39, 53, 64]. Electrical sensitivity has also been used and the parameters influencing it have been thoroughly studied by psychologists in the last decades [50,128]. Precise control of stimulus intensity can be achieved easily, and absolute thresholds of electrical stimulation determined. Electricity has also been used in the study of pain sensitivity [93]. For the quantitative study of light touch sensitivity, an apparatus that overcomes the difficulties encountered with Von Frey hairs has been devised [34]. It consists of a probe mounted on a motor driven by a function generator. A system for the quantification of vibration sensitivity will be described later and the advantages offered by vibratory stimulation will be discussed.

TABLE 1

Drugs	Therapeutic Use	Sensory Signs and Symptoms	References
Vincristine (Oncovin)	antitumor	impaired touch, pinprick, vibration sense, paresthesia, numbness, tingling	13, 15, 27, 85, 88, 90, 103, 105, 108, 110, 136
Vinblastine (Velban)	antitumor	paresthesia	115
Vindesine (NSC-245467)	antitumor	paresthesia	138
Cis-diamminedichloroplatinum (Platinal)	antitumor	impaired vibration, touch, pinprick sense, paresthesia, numbness, tingling	10, 67
Maytansine (NSC-153858)	antitumor	paresthesia	9, 95
Methylhydrazine (Procarbazine)	antitumor	paresthesia, sensorium changes	109
Podophyllum resin (Podophyllin)	antitumor	paresthesia, generalized hypesthesia	18, 24
Misonidazole (Ro-07-0582)	hypoxic cell radiosensitizer	paresthesia, reduced pinprick, light touch, vibration and temperature sensitivity, coldness	30, 130
Metronidazole (Flagyl)	antimicrobial	paresthesia, hyperesthesia, decreased touch, pinprick and temperature sense	28, 101, 107, 129
Nitrofurantoin (Furadantin)	antimicrobial	numbness, paresthesia, tingling, dysesthesia, sensory loss (pinprick, pain vibration, temperature, light touch)	35, 52, 63, 80, 83 106, 124
Nitrofurazone (Furacin)	antimicrobial	numbness, paresthesia, impaired touch, pain and vibration sense	26
Furaltadone (Altafur)	antimicrobial	loss of all sensory modalities	26, 61
Thiophenicol (Thiomycetin)	antimicrobial	tingling, burning, tactile hypesthesia, dysesthesia, decreased vibration sense	114
Chloramphenicol (Chloromycetin)	antimicrobial	numbness, tingling, burning, decreased touch, vibration sense	20, 65
Iodochlorhydroxyquin (Entero-Vioform)	antimicrobial	numbness, tingling, dysesthesia, impaired touch, temperature, pain and vibration sense	117, 120, 126
Demeclocycline (Declomycin)	antimicrobial	paresthesia, tingling, burning sensation	37
Doxycycline (Vibramycin)	antimicrobial	paresthesia, tingling	38
Colistimethate (Colistin)	antimicrobial	paresthesia	69
Dapsone (Avlosulfon)	antimicrobial	numbness, decreased light touch, pain, two-point discrimination, vibration sense	36, 70
Ammoniated mercury ointment	antiseptic	decreased pain and vibration sense, numbness	123
Methylmercury thioacetamide	fungicidal	numbness, sensory disturbance	96
Gold thiomalate (Myochrysin)	antarthritic	tingling, pricking, paresthesia, decreased touch, temperature, pain and vibration sense, hyperesthesia, burning, pins and needles sensations	32, 78
Phenytoin (Dilantin)	antiepileptic	dysesthesia, numbness, tingling, decreased pain and vibration sense	31, 81
Nitrous oxide	anesthetic	numbness, paresthesia, dysesthesia, tingling, impaired touch and vibration	73, 74
Perhexiline maleate (Pexid)	antianginal	dysesthesia, paresthesia, impaired deep sensitivity, decreased touch, pain, temperature and vibration sense	2, 11, 75, 79
Disulfiram (Antabuse)	antialcohol	tingling, numbness, dysesthesia, cold or burning sensation, impaired pinprick, touch, vibration and temperature sense	6, 12, 21, 44, 48, 89
Isoniazid (Niconyl)	antituberculous	tingling, numbness, pins and needles, electric shock sensation, burning pain, dysesthesia, impaired vibration, temperature, touch and two-point discrimination, hyperalgesia	8, 42, 94
Ethionamide (Trecator)	antituberculous	numbness, impaired vibration sense, dysesthesia, burning sensation, hyperalgesia, impaired vibration sense	100
Hydralazine (Apresoline)	antihypertensive	pins and needles, numbness	102
Thalidomide (Distaval)	sedative	paresthesia, numbness, hyperalgesia, impaired light touch, vibration sense	40, 41
Furosemide (Lasix)	diuretic	burning paresthesia	84

TABLE 2

Chemicals	Signs and Symptoms	References
Acrylamide	decreased vibration, temperature, touch and pinprick, numbness, paresthesia, coldness, tingling, pins and needles	17, 45, 49
Triorthocresyl phosphate	paresthesia, numbness, formication, dysesthesia, aching pain, impaired touch, temperature, pain and vibration sense, coldness	7, 14, 46, 87, 116, 122, 134
n-Hexane	paresthesia, numbness, burning sensation or coldness, tingling, dysesthesia, decreased pinprick, touch, temperature and vibration sense	23, 47, 56, 71, 99, 104, 111, 125
Methyl n-butyl ketone	numbness, impaired pain, light touch, temperature and vibration sense	86, 137
Carbon disulfide	numbness, paresthesia, tingling, decreased touch, temperature and pain sensitivity	68, 131
Arsenic	numbness, tingling, burning sensation, paresthesia, dysesthesia, decreased touch, temperature and vibration sense, formication, hyperalgesia, hyperesthesia	22, 43, 55, 57, 77, 112
Mercury	paresthesia, numbness, tingling, sensation of needle-pricking, superficial and deep sensory disturbance (touch, pain, two-point discrimination, vibration)	1, 3, 54, 59, 60, 76, 82, 97, 127, 135
Thallium	paresthesia, dysesthesia, tingling, burning pain, numbness, hyperalgesia, impaired light touch, pinprick and vibration sense, hyperesthesia	4, 16, 51, 62
Lead	paresthesia	29, 113
Cyanide	paresthesia, numbness, pins and needles, burning, aching pain, feeling of heat and cold, decreased two-point discrimination, pinprick, light touch, temperature and vibration sense	98
Chlorobiphenyl	numbness, decreased temperature, pain and touch sensitivity	92
Methyl bromide	numbness, decreased vibration sense, impaired superficial sensation in all modalities	66

CHEMICALS AS ETIOLOGIC AGENTS IN SENSORY DISORDERS

A wide variety of drugs and toxic chemicals of unrelated structures and modes of action can induce sensory disorders of peripheral or central origin. Drugs with unrelated therapeutic actions have been implicated in the development of peripheral neuropathy mainly characterized by sensory disorders. The main sensory signs and symptoms due to selected drugs are shown in Table 1.

Several groups of anticancer drugs have neurotoxic peripheral side effects which may be severe enough to limit therapeutic treatment. Of the vinca alkaloids, this is particularly the case with vincristine. Hypoxic cell sensitizers, such as misonidazole and metronidazole, which are also clinical antiflagellates, are used as adjuvants to radiotherapy and also produce neurotoxicity.

Derivatives of the furan family, which possess antibacterial activity, are also radiosensitizers of hypoxic mammalian cells [19]. Nitrofurantoin neurotoxicity is a problem especially in patients with severe impairment of renal function, or when the drug is administered in high doses for prolonged periods of time. Various antibiotics of the chloramphenicol, oxyquinoline, tetracycline and polymyxin families can induce neurotoxic peripheral side effects in a small susceptible fraction of patients, as is also the case with some drugs of the hydantoin and sulfone families. Polyneuropathy with early sensory complaints is seen in subjects exposed to nitrous oxide and is also a known entity in chronic alcoholism. However, there is evidence that hypovitaminosis, rather than alcohol per se, is largely responsible for this effect. Control of alcoholism with disulfiram has been associated with peripheral neuropathy and optic neuritis although their occurrence has not been widely recognized. More common are the sensory side effects associated with antituberculous therapy, especially with isoniazid. There have also been reports of iatrogenic neuropathy induced by some antianginal agents, fungicides, antiseptics, antarthritics, sedatives and diuretics, which seem to produce fleeting peripheral paresthesias and other sensory changes.

Besides drugs, many industrial and environmental toxic chemicals have contributed significantly to clinical peripheral nerve dysfunction (Table 2). Here, too, chemicals of completely unrelated structures and modes of action can present similar neurological profiles. In acrylamide toxicity, sensory signs and symptoms precede and predominate over motor disturbances. Triorthocresyl phosphate, which is used as a plasticizer, has been the origin of several repeated outbreaks in Morocco, where thousands of people were affected by adulterated cooking oil. India (with contaminated mustard oil) and the United States (with Jamaica ginger) have not been spared. More or less important sensory involvement

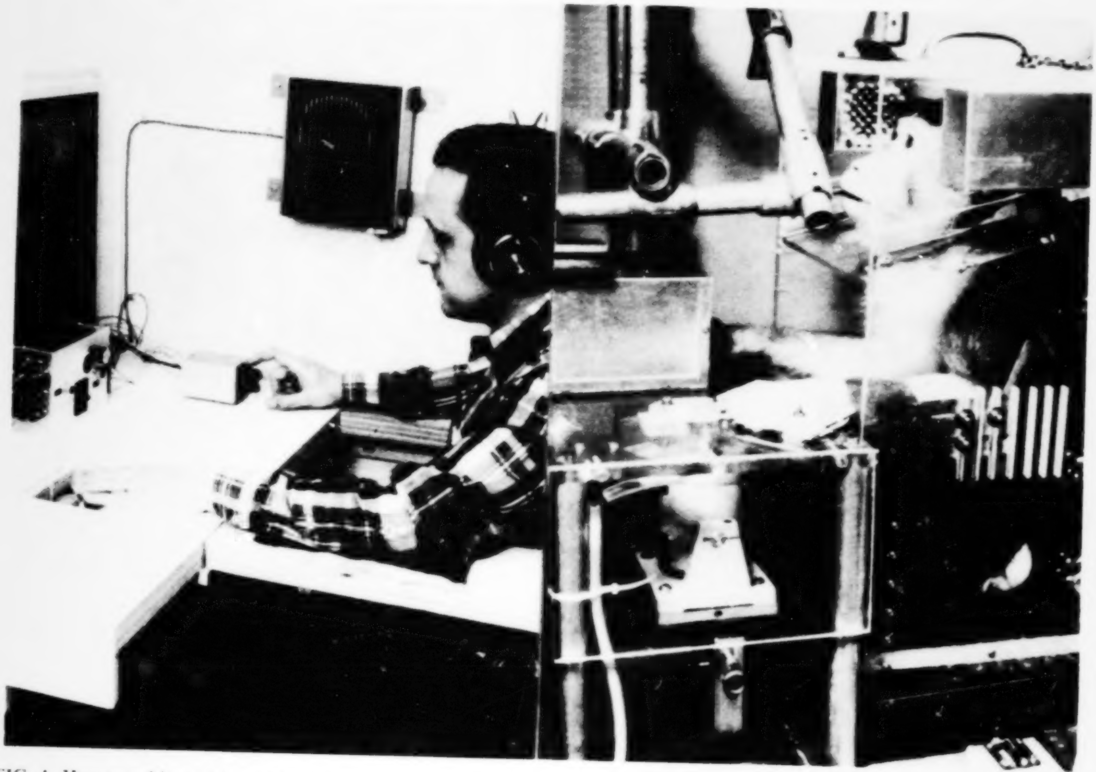

FIG. 1. Human subject and monkey during a testing session.

has characterized these outbreaks. Several organic solvents and heavy metals share in common adverse effects on the somatosensory system. Such effects are protean and range from subjective numbness and tingling to objective cutaneous sensory loss. Other industrial chemicals have similar effects. Dietary cyanide has instigated outbreaks of sensory ataxia mainly in tropical and subtropical regions, where the staple food is cassava, very rich in cyanogenetic glycosides.

VIBRATION SENSITIVITY

Anatomical and Functional Substrates

Vibration sensitivity is mediated by a duplex mechanism. Psychophysical evidence for this claim is based on the shape of the function relating vibration frequency to the just detectable vibration amplitude (absolute threshold). Below about 50 Hz, threshold is independent of frequency. Above this value, there is a frequency dependent decrease in threshold with an optimal sensitivity around 250 Hz. Then, the amplitude-frequency detection curve rises. It was concluded, from this function, that temporal summation occurs above 50 Hz, and is absent below this point. Increasing the size of the vibrating contactor results in a lower threshold at higher frequencies while it does not affect thresholds in the low frequency range. This is evidence for a spatial summation selective for higher frequencies [132,133].

Electrophysiological data also support the idea that vibration sensitivity is mediated by a pluralistic mechanism and depends on at least two sets of thick myelinated nerve fibers and receptors. One set is thought to end in Pacinian corpuscles and has its tuning points and absolute thresholds of afferent discharge at frequencies higher than 50 Hz. Another set has a maximal sensitivity in the low frequency range. Its anatomical substrate has not been convincingly identified yet, although Meissner's corpuscles have been proposed [91].

Correlation with Subjective Symptoms

With most of the compounds cited in Tables 1 and 2, subjective sensory symptoms, such as distal paresthesia, numbness and tingling, are often early manifestations of neurotoxicity and are followed by objective sensory dysfunction. No systematic study has been done to correlate subjective symptoms with objective signs. However, in several clinical case reports, where vibration was measured with quantitative and objective methods, it was mentioned that numbness occurred concurrently with vibration sensitivity impairment [25,72]. Paresthesias tended to confuse the results of the examination of vibratory sensitivity [5,121].

We do not believe, however, that every subjective complaint must necessarily be accompanied by vibration sensitivity impairment. Some chemicals can be more or less selective for some types of nerve fibers. Thalidomide, for example, preferentially affects large diameter nerve fibers

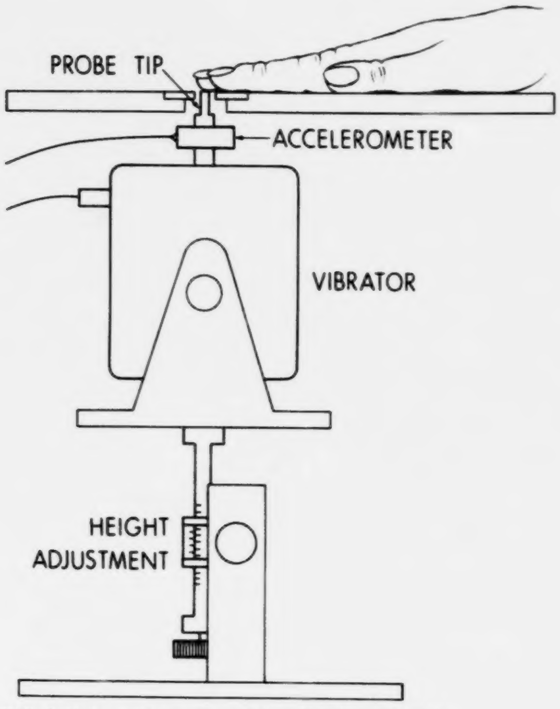

FIG. 2. Schematic drawing of the vibration assembly.

[41]. This is also the case for acrylamide [58], which also inactivates mechanoreceptive properties of Pacinian corpuscles before any alteration of ultrastructural features of the nerve fiber or the sensory terminal [118,119]. Disulfiram damages myelinated fibers and spares unmyelinated fibers [89]. Vincristine causes loss of both small and large diameter fibers [85]. A drug that would selectively affect unmyelinated fibers or thin myelinated fibers would certainly have a minimal effect on vibration sensitivity, which is mainly carried through thick myelinated fibers. Pain and temperature sensitivity, on the other hand, might be more significantly impaired.

Methods and Preliminary Data

Vibration sensitivity can be determined objectively and accurately in human and nonhuman primates with essentially the same methods and equipment. The monkey sits in a restraining chair in front of a small table (Fig. 1). The left hand is immobilized in a plasticene mold. The tip of the middle finger is placed on the vibrating probe, which protrudes through a hole (Fig. 2). The position of the vibrator can be adjusted with a precision gear apparatus so as to indent the skin by a constant depth when the probe makes electrical contact with the finger. The right hand is free and has access to a telegraph key. A spout next to the monkey's mouth delivers fruit juice when an electromagnetic valve is activated. Two loudspeakers are located at the top of the restraining chair. The whole system is enclosed in a sound-attenuated double-walled chamber. A white noise generator is turned on during the testing session.

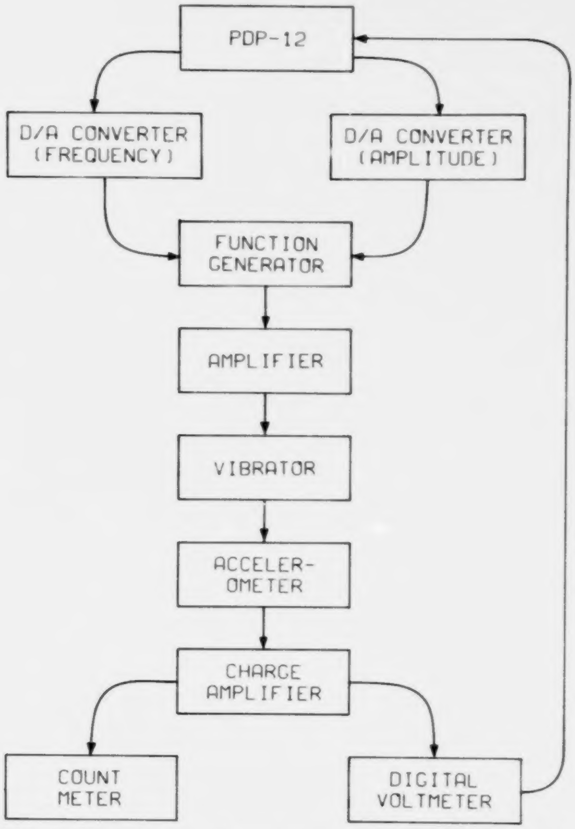

FIG. 3. Block diagram of arrangement for stimulus delivery and measurement.

Similarly, the human subject sits on a chair facing a table. Instruction is given to keep the left hand in a relaxed position with the tip of the middle finger on the vibrating contactor. The subject wears headphones during the session. The right hand has access to a telegraph key. Performance feedback is given through an intelligence panel. Verbal instructions are given to the subject about the task requested.

Sinusoidal vibrations are generated through the system illustrated in Fig. 3. The whole experiment, including stimulus presentation and data collection, is under complete control of an on-line PDP-12 computer (Digital Equipment Corporation). Frequency and amplitudes are independently controlled with a function generator whose output is amplified and drives the vibrator. On the moving shaft is mounted a piezoelectric accelerometer which permits accurate measurement of vibration amplitude.

The testing session is divided into discrete trials. A tone is turned on. The subject then presses the key, holds it down, and, after a variable interval, vibration is delivered to the finger. A key release during vibration is rewarded either by fruit juice (monkey) or by addition of a point to a counter (human). In order to get a quantitative estimate of guessing bias, catch trials are randomly introduced during the session. They differ from normal trials in that no vibration is presented. The trial starts with the tone onset, the subject de-

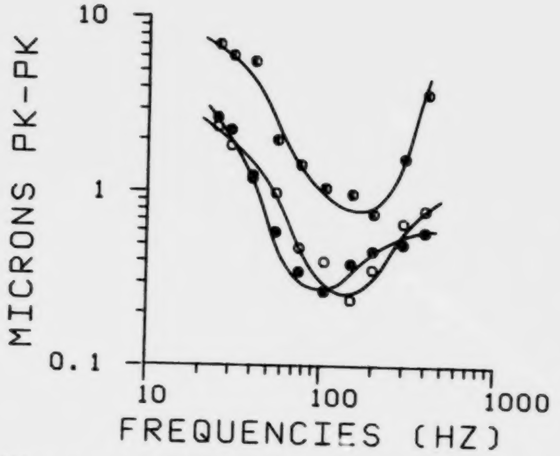

FIG. 4. Normal vibration thresholds in monkey and human subject. Empty and closed circles show two independent determinations of thresholds. Half closed circles represent the performance of a human subject.

presses the key and holds it down. After a variable interval, the tone is turned off. Key release at that time is also rewarded.

Vibratory stimuli are presented according to the up-and-down method. When the subject is able to detect vibration, the amplitude of the next vibration is decreased in steps until the subject cannot detect the stimulus any more, in which case its amplitude is subsequently increased.

With the equipment and methods described, it has been possible to measure vibration sensitivity at different frequencies. Normative data for one monkey and one human subject can be seen in Fig. 4. The curves with the open and closed circles represent absolute thresholds of vibration sensitivity obtained in one monkey about two months apart. A remarkable stability characterizes the monkey's performance. Human data are given to show the similarities between human and nonhuman primates.

CONCLUSIONS

Monkeys studied with the technique described here represent a very good model of human vibration sensitivity. Anatomical and physiological similarities account for the agreement observed in cutaneous sensitivity of human and nonhuman primates. Application of this model to toxicity studies is underway, and we believe that these studies are likely to provide meaningful data applicable to the human situation. This approach has also the advantage of being comparable to audition and vision, where the frequency dimension has proven to be an important parameter in the prediction of toxicity.

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REFERENCES

1. Ahlmark, A. Poisoning by methyl mercury compounds. *Br. J. ind. Med.* **5:** 117–119, 1948.
2. Augustin, P., M. Samson and L. Verdure. A propos d'une observation de polyneuropathie due au maléate de perhexiline. *Rev. Oto-Neuro-Ophthal.* **49:** 133–139, 1977.
3. Bakir, F., S. F. Damluji, L. Amin-Zaki, M. Murtadha, A. Khalidi, N. Y. Al-Rawi, S. Tikriti, H. I. Dhahir, T. W. Clarkson, J. C. Smith and R. A. Doherty. Methylmercury poisoning in Iraq. *Science* **181:** 230–241, 1973.
4. Bank, W. J., D. E. Pleasure, K. Suzuki, M. Nigro and R. Katz. Thallium poisoning. *Archs Neurol.* **26:** 456–464, 1972.
5. Barach, J. H. Test for quantitative vibratory sensation in diabetes, pernicious anemia and tabes dorsalis. *Archs int. Med.* **79:** 602–613, 1947.
6. Barry, W. K. Peripheral neuritis following tetraethylthiuram disulphide treatment. *Lancet* **2:** 937, 1953.
7. Bennett, C. R. A group of patients suffering from paralysis due to drinking Jamaica ginger. *Sth med. J., Birmingham* **23:** 371–375, 1930.
8. Biehl, J. P. and J. H. Skavlem. Toxicity of isoniazid. *Am. Rev. Tuberc. pulm. Dis.* **68:** 296–297, 1953.
9. Blum, R. H. and T. Kahlert. Maytansine: a phase I study of an ansa macrolide with antitumor activity. *Cancer Treat Rep.* **62:** 435–438, 1978.
10. Bonomi, P. D., R. E. Slayton and J. Wolter. Phase II trial of adriamycin and *cis*-dichlorodiammineplatinum (II) in squamous cell, ovarian, and testicular carcinomas. *Cancer Treat. Rep.* **62:** 1211–1213, 1978.
11. Bourrat, C., J. J. Viala and J. P. Guastala. Neuropathie périphérique après absorption prolongée de maléate de perhexiline. *Nouvelle Presse médicale* **4:** 2528, 1975.
12. Bradley, W. G. and R. L. Hewer. Peripheral neuropathy due to disulfiram. *Br. med. J.* **2:** 449–450, 1966.
13. Bradley, W. G., L. P. Lassman, G. W. Pearce and J. N. Walton. The neuromyopathy of vincristine in man. Clinical, electrophysiological and pathological studies. *J. neurol. Sci.* **10:** 107–131, 1970.
14. Burley, B. T. Polyneuritis from tricresyl phosphate. *J. Am. med. Ass.* **98:** 298–304, 1932.
15. Casey, E. B., A. M. Jeliffe, P. M. Le Quesne and Y. L. Millett. Vincristine neuropathy. Clinical and electrophysiological observations. *Brain* **96:** 69–86, 1973.
16. Cavanagh, J. B., N. H. Fuller, H. R. M. Johnson and P. Rudge. The effects of thallium salts, with particular reference to the nervous system changes. *Q. J. Med.* **43:** 293–319, 1974.
17. Cavigneaux, A. and G. B. Cabasson. Intoxication par l'acrylamide. *Archs Mal. prof.* **33:** 115–116, 1972.
18. Chamberlain, M. J., A. L. Reynolds and W. B. Yeoman. Toxic effect of podophyllum application in pregnancy. *Br. med. J.* **3:** 391–392, 1972.
19. Chapman, J. D., A. P. Reuvers, J. Borsa, A. Petkau and D. R. McCalla. Nitrofurans as radiosensitizers of hypoxic mammalian cells. *Cancer Res.* **32:** 2616–2624, 1972.

20. Charache, S., D. Finkelstein, P. S. Lietman and J. Scott. Peripheral and optic neuritis in a patient with hemoglobin SC disease during treatment of Salmonella osteomyelitis with chloramphenicol. *Johns Hopkins med. J.* **140:** 121–124, 1977.
21. Charatan, F. B. Peripheral neuritis following tetraethylthiuram disulphide treatment. *Br. med. J.* **2:** 380, 1953.
22. Chhuttani, P. N., L. S. Chawla and T. D. Sharma. Arsenical neuropathy. *Neurology, Minneap.* **17:** 269–274, 1967.
23. Cianchetti, C., G. Abbritti, G. Perticoni, A. Siracusa and F. Curradi. Toxic polyneuropathy of shoe-industry workers. A study of 122 cases. *J. Neurol. Neurosurg. Psychiat.* **39:** 1151–1161, 1976.
24. Clark, A. N. G. and M. J. Parsonage. Case of podophyllum poisoning with involvement of the nervous system. *Br. med. J.* **2:** 1155–1157, 1957.
25. Collens, W. S., J. D. Zilinsky and L. C. Boas. Quantitative estimation of vibratory sense as a guide for treatment of peripheral neuritis in diabetes. *Proc. Am. Diabetes Ass.* **6:** 459–468, 1946.
26. Collings, H. Polyneuropathy associated with nitrofuran therapy. *Archs Neurol., Chicago* **3:** 656–660, 1960.
27. Costa, G., M. M. Hreshchyshyn and J. F. Holland. Initial clinical studies with vincristine. *Cancer Chemother. Rep.* **24:** 39–44, 1962.
28. Coxon, A. and C. A. Pallis. Metronidazole neuropathy. *J. Neurol. Neurosurg. Psychiat.* **39:** 403–405, 1976.
29. Dagnino, N. and R. Badino. Polineuropatia da saturnismo idrico. *Sist. nerv.* **20:** 417–420, 1968.
30. Dische, S., M. I. Saunders, M. E. Lee, G. E. Adams and I. R. Flockhart. Clinical testing of the radiosensitizer Ro 07-0582: experience with multiple doses. *Br. J. Cancer* **35:** 567–579, 1977.
31. Dobkin, B. H. Reversible subacute peripheral neuropathy induced by phenytoin. *Archs Neurol., Chicago* **34:** 189–190, 1977.
32. Doyle, J. B. and E. F. Cannon. Severe polyneuritis following gold therapy for rheumatoid arthritis. *Ann. int. Med.* **33:** 1468–1472, 1950.
33. Dyck, P. J., D. J. Curtis, W. Bushek and K. Offord. Description of "Minnesota Thermal Disks" and normal values of cutaneous thermal discrimination in man. *Neurology, Minneap.* **24:** 325–330, 1974.
34. Dyck, P. J., E. H. Lambert and P. C. Nichols. Quantitative measurement of sensation related to compound action potential and number and sizes of myelinated and unmyelinated fibers of sural nerve in health, Freidreich's ataxia, hereditary sensory neuropathy, and tabes dorsalis. In: *Handbook of Electroencephalography and Clinical Neurophysiology*. Amsterdam: Elsevier Publishing Co., Vol. 9, 1971, pp. 83–118.
35. Ellis, F. G. Acute polyneuritis after nitrofurantoin therapy. *Lancet* **2:** 1136–1138, 1962.
36. Epstein, F. W. Dapsone-induced peripheral neuropathy. *Archs Derm.* **112:** 1761–1762, 1976.
37. Frost, P., G. D. Weinstein and E. C. Gomez. Methacycline and demeclocycline in relation to sunlight. *J. Am. med. Ass.* **216:** 326–329, 1971.
38. Frost, P., G. D. Weinstein and E. C. Gomez. Phototoxic potential of minocycline and doxycycline. *Archs Derm.* **105:** 681–683, 1972.
39. Fruhstorfer, H., U. Lindblom and W. G. Schmidt. Method for quantitative estimation of thermal thresholds in patients. *J. Neurol. Neurosurg. Psychiat.* **39:** 1071–1075, 1976.
40. Fullerton, P. M. and M. Kremer. Neuropathy after intake of thalidomide (Distaval). *Br. med. J.* **2:** 855–858, 1961.
41. Fullerton, P. M. and D. J. O'Sullivan. Thalidomide neuropathy: a clinical, electrophysiological, and histological follow-up study. *J. Neurol. Neurosurg. Psychiat.* **31:** 543–551, 1968.
42. Gammon, G. D., F. W. Burge and G. King. Neural toxicity in tuberculous patients treated with isoniazid (isonicotinic acid hydrazide). *Archs. Neurol. Psychiat., Chicago* **70:** 64–69, 1953.
43. Garb, L. G. and C. H. Hine. Arsenical neuropathy: residual effects following acute industrial exposure. *J. occup. Med.* **19:** 567–568, 1977.
44. Gardner-Thorpe, C. and S. Benjamin. Peripheral neuropathy after disulfiram administration. *J. Neurol. Neurosurg. Psychiat.* **34:** 253–259, 1971.
45. Garland, T. O. and M. W. H. Patterson. Six cases of acrylamide poisoning. *Br. med. J.* **4:** 134–138, 1967.
46. Geoffroy, H., A. Slomic, M. Benenadji and P. Pascal. Myelo-polynévrites tri-crésyl phosphatées. Toxi-épidémie marocaine de septembre-octobre 1959. *Wld Neurol.* **1:** 294–315, 1960.
47. Goto, I., M. Matsumura, N. Inoue, Y. Murai, K. Shida, T. Santa and Y. Kuroiwa. Toxic polyneuropathy due to glue sniffing. *J. Neurol. Neurosurg. Psychiat.* **37:** 848–853, 1974.
48. Graveleau, J. Les neuropathies périphériques du disulfirame (Antabuse). *Rev. neurol.* **126:** 149–153, 1972.
49. Graveleau, J., P. Loirat and V. Nusinovici. Polynévrite par l'acrylamide. *Rev. neurol.* **123:** 62–65, 1970.
50. Green, R. T. The absolute threshold of electric shock. *Br. J. Psychol.* **53:** 107–115, 1962.
51. Grunfeld, O. and G. Hinostroza. Thallium poisoning. *Archs int. Med.* **114:** 132–138, 1964.
52. Hakamies, L. and M. Mumenthaler. Particolarità della polineuropatia da nitrofurantoina. *Minerva med., Roma* **64:** 2624–2626, 1973.
53. Hamann, H. D., H. O. Handwerker and H. Assmus. Quantitative assessment of altered thermal sensation in patients suffering from cutaneous nerve disorders. *Neuroscience Lett.* **9:** 273–277, 1978.
54. Harada, M. Minamata disease. Chronology and medical report. *Bull. Inst. constitutional Med. Kumamoto* **25:** 1–60, 1976.
55. Hassin, G. Symptomatology of arsenical polyneuritis. *J. nerv. ment. Dis.* **72:** 628–636, 1930.
56. Herskowitz, A., N. Ishii and H. Schaumburg. n-Hexane neuropathy. A syndrome occurring as a result of industrial exposure. *New Engl. J. Med.* **285:** 82–85, 1971.
57. Heyman, A., J. B. Pfeiffer, R. W. Willett and H. M. Taylor. Peripheral neuropathy caused by arsenical intoxication. A study of 41 cases with observations on the effect of BAL (2,3, dimercapto-propanol). *New Engl. J. Med.* **254:** 401–409, 1956.
58. Hopkins, A. P. and R. W. Gilliatt. Motor and sensory nerve conduction velocity in the baboon: normal values and changes during acrylamide neuropathy. *J. Neurol. Neurosurg. Psychiat.* **34:** 415–426, 1971.
59. Hunter, D., R. R. Bomford and D. S. Russell. Poisoning by methyl mercury compounds. *Q. J. Med.* **9:** 193–213, 1940.
60. Hunter, D. and D. S. Russell. Focal cerebral and cerebellar atrophy in a human subject due to organic mercury compounds. *J. Neurol. Neurosurg. Psychiat.* **17:** 235–241, 1954.
61. Hussain, K. K. and H. Koilpillai. Toxic effects of furaltadone. *Lancet* **2:** 490, 1960.
62. Innis, R. and H. Moses. Thallium poisoning. *Johns Hopkins med. J.* **142:** 27–31, 1978.
63. Jacknowitz, A. I., J. L. Le Frock and R. A. Prince. Nitrofurantoin polyneuropathy: report of two cases. *Am. J. Hosp. Pharm.* **34:** 759–762, 1977.
64. Jones, N., P. A. Twelker and D. Singer. A waterless thermal stimulator. *Am. J. Psychol.* **75:** 147–149, 1962.
65. Joy, R. J. T., R. Scalettar and D. B. Sodee. Optic and peripheral neuritis. Probable effects of prolonged chloramphenicol therapy. *J. Am. med. Ass.* **173:** 1731–1734, 1960.
66. Kantarjian, A. D. and A. S. Shaheen. Methyl bromide poisoning with nervous system manifestations resembling polyneuropathy. *Neurology, Minneap.* **13:** 1054–1068, 1963.
67. Kedar, A., M. E. Cohen and A. I. Freeman. Peripheral neuropathy as a complication of *cis*-dichlorodiammineplatinum (II) treatment: a case report. *Cancer Treat. Rep.* **62:** 819–821, 1978.
68. Knave, B., B. Kolmodin-Hedman, H. E. Persson and J. M. Goldberg. Chronic exposure to carbon disulfide: effects on occupationally exposed workers with special reference to the nervous system. *Wk-Environment-Hlth* **11:** 49–58, 1974.

69. Koch-Weser, J., V. W. Sidel, E. B. Fereman, P. Kanarek, D. C. Finer and A. E. Eaton. Adverse effects of sodium colistimethate. Manifestations and specific reaction rates during 317 courses of therapy. *Ann. int. Med.* **72:** 857–868, 1970.

70. Koller, W. C., L. K. Gehlmann, F. D. Malkinson and F. A. Davis. Dapsone-induced peripheral neuropathy. *Archs Neurol., Chicago* **34:** 644–646, 1977.

71. Korobkin, R., A. K. Asbury, A. J. Sumner and S. L. Nielsen. Glue-sniffing neuropathy. *Archs Neurol., Chicago* **32:** 158–162, 1975.

72. Laidlaw, R. W., M. A. Hamilton and R. M. Brickner. The occurrence of dissociated disturbances of pallesthesia and kinesthesia. *Bull. neurol. Inst. N.Y.* **7:** 303–320, 1938.

73. Layzer, R. B. Myeloneuropathy after prolonged exposure to nitrous oxide. *Lancet* **2:** 1227–1230, 1978.

74. Layzer, R. B., R. A. Fishman and J. A. Schafer. Neuropathy following abuse of nitrous oxide. *Neurology, Minneap.* **28:** 504–506, 1978.

75. Le Menn, G., D. Mabin and P. Penther. Régression lente et incomplète d'une neuropathie périphérique due au maléate de perhexiline. *Ann. Cardiol. Angéiol.* **26:** 149–150, 1977.

76. Le Quesne, P. M., S. F. Damluji and H. Rustam. Electrophysiological studies of peripheral nerves in patients with organic mercury poisoning. *J. Neurol. Neurosurg. Psychiat.* **37:** 333–339, 1974.

77. Le Quesne, P. M. and J. G. MacLeod. Peripheral neuropathy following a single exposure to arsenic. *J. neurol. Sci.* **32:** 437–451, 1977.

78. Lescher, F. G. Nervous complication following treatment by gold salts. *Br. med. J.* **2:** 1303–1305, 1936.

79. Lhermitte, F., M. Fardeau, F. Chedru and J. Mallecourt. Polyneuropathy after perhexiline maleate therapy. *Br. med. J.* **1:** 1256, 1976.

80. Loughridge, L. W. Peripheral neuropathy due to nitrofurantoin. *Lancet* **2:** 1133–1135, 1962.

81. Lovelace, R. E. and S. J. Horwitz. Peripheral neuropathy in long-term diphenylhydantoin therapy. *Archs Neurol., Chicago* **18:** 69–77, 1968.

82. Maghazaji, H. I. Psychiatric aspects of methylmercury poisoning. *J. Neurol. Neurosurg. Psychiat.* **37:** 954–958, 1974.

83. Martin, W. J., K. B. Corbin and D. C. Utz. Paresthesias during treatment with nitrofurantoin: report of a case. *Proc. Mayo Clin.* **37:** 288–292, 1962.

84. Materson, B. J. Generalized burning paresthesia due to intravenous furosemide. *J. Fla med. Ass.* **58:** 34–35, 1971.

85. McLeod, J. G. and R. Penny. Vincristine neuropathy: an electrophysiological and histological study. *J. Neurol. Neurosurg. Psychiat.* **32:** 297–304, 1969.

86. Mendell, J. R., K. Saida, M. F. Ganansia, D. B. Jackson, H. Weiss, R. W. Gardier, C. Chrisman, N. Allen, D. Couri, J. O'Neill, B. Marks and L. Hetland. Toxic polyneuropathy produced by methyl n-butyl ketone. *Science* **185:** 787–789, 1974.

87. Merritt, H. H. and M. Moore. Peripheral neuritis associated with ginger extract ingestion. *New Engl. J. Med.* **203:** 4–12, 1930.

88. Mittelman, A., R. Grindberg and T. Dao. Clinical experience with vincristine (NSC-67574) in advanced cancer of the breast. *Cancer Chemother. Rep.* **34:** 25–30, 1964.

89. Moddel, G., J. M. Bilbao, D. Payne and P. Ashby. Disulfiram neuropathy. *Archs Neurol., Chicago* **35:** 658–660, 1978.

90. Moress, G. R., A. N. D'Agostino and L. W. Jarcho. Neuropathy in lymphoblastic leukemia treated with vincristine. *Archs Neurol., Chicago* **16:** 377–384, 1967.

91. Mountcastle, V. B., R. H. LaMotte and G. Carli. Detection thresholds for stimuli in humans and monkeys: comparison with threshold events in mechanoreceptive afferent nerve fibers innervating the monkey hand. *J. Neurophysiol.* **35:** 122–136, 1972.

92. Murai, Y. and Y. Kuroiwa. Peripheral neuropathy in chlorobiphenyl poisoning. *Neurology, Minneap.* **21:** 1173–1176, 1971.

93. Notermans, S. L. H. Measurement of the pain threshold determined by electrical stimulation and its clinical application. Part II. Clinical application in neurological and neurosurgical patients. *Neurology, Minneap.* **17:** 58–73, 1967.

94. Ochoa, J. Isoniazid neuropathy in man: quantitative electron microscope study. *Brain* **93:** 831–850, 1970.

95. O'Connell, M. J., A. Shani, J. Rubin and C. G. Moertel. Phase II trial of maytansine in patients with advanced colorectal carcinoma. *Cancer Treat. Rep.* **62:** 1237–1238, 1978.

96. Okinaka, S., M. Yoshikawa, T. Mozai, Y. Mizuno, T. Terao, H. Watanabe, K. Ogihara, S. Hirai, Y. Yoshino, T. Inose, S. Anzai and M. Tsuda. Encephalomyelopathy due to an organic mercury compound. *Neurology* **14:** 69–76, 1964.

97. Ordóñez, J. V., J. A. Carrillo, M. Miranda and J. L. Gale. Estudio epidemiologico de una enfermedad considerada como encefalitis en la region de los altos de Guatemala. *Boln Of. sanit. pan-am.* **60:** 510–519, 1966.

98. Osuntokun, B. O. An ataxic neuropathy in Nigeria. A clinical, biochemical and electrophysiological study. *Brain* **91:** 215–248, 1968.

99. Paulson, G. W. and G. W. Waylonis. Polyneuropathy due to n-hexane. *Archs int. Med.* **136:** 880–882, 1976.

100. Poole, G. W. and J. Schneeweiss. Peripheral neuropathy due to ethionamide. *Am. Rev. resp. Dis.* **84:** 890–892, 1961.

101. Ramsay, I. D. Endocrine ophthalmology. *Br. med. J.* **4:** 706, 1968.

102. Raskin, N. H. and R. A. Fishman. Pyridoxine-deficiency neuropathy due to hydralazine. *New Engl. J. Med.* **273:** 1182–1185, 1965.

103. Reitemeier, R. J., C. G. Moertel and C. M. Blackburn. Vincristine (NSC-67574) therapy of adult patients with solid tumors. *Cancer Chemother. Rep.* **34:** 21–23, 1964.

104. Rizzuto, N., H. Terzian and S. Galiazzo-Rizzuto. Toxic polyneuropathies in Italy due to leather cement poisoning in shoe industries. A light- and electron-microscopic study. *J. neurol. Sci.* **31:** 343–354, 1977.

105. Rosenthal, S. and S. Kaufman. Vincristine neurotoxicity. *Ann. int. Med.* **80:** 733–737, 1974.

106. Rubenstein, C. J. Peripheral polyneuropathy caused by nitrofurantoin. *J. Am. med. Ass.* **187:** 647–649, 1964.

107. Said, G., J. Goasguen and C. Laverdant. Polynévrites au cours des traitements prolongés par le métronidazole. *Rev. neurol.* **134:** 515–521, 1978.

108. Sakamoto, A. Physical activity: a possible determinant of vincristine (NSC-67574) neuropathy. *Cancer Chemother. Rep.* **58:** 413–415, 1974.

109. Samuel, M. L., W. V. Leary, R. Alexanian, C. D. Howe and E. Frei III. Clinical trials with N-isopropyl-(2-methylhydrazino)-p-toluamide hydrochloride in malignant disseminated neoplasia. *Cancer* **20:** 1187–1194, 1967.

110. Sandler, S. G., W. Tobin and E. S. Henderson. Vincristine-induced neuropathy. A clinical study of fifty leukemic patients. *Neurology, Minneap.* **19:** 367–374, 1969.

111. Scala, R. A. Hydrocarbon neuropathy. *Ann. occup. Hyg.* **19:** 293–299, 1976.

112. Senanayake, N., W. A. S. de Silva and M. S. L. Salgado. Arsenical neuropathy—A clinical study. *Ceylon med. J.* **17:** 195–203, 1972.

113. Seppäläinen, A. M. and S. Hernberg. Sensitive technique for detecting subclinical lead neuropathy. *Br. J. ind. Med.* **29:** 443–449, 1972.

114. Shinohara, Y., F. Yamaguchi and F. Gotoh. Toxic neuropathy as a complication of thiophenicol therapy. *Eur. Neurol.* **16:** 161–164, 1977.

115. Smart, C. R., D. B. Rochlin, A. M. Nahum, A. Silva and D. Wagner. Clinical experience with vinblastine sulfate (NSC-49842) in squamous cell carcinoma and other malignancies. *Cancer Chemother. Rep.* **34:** 31–45, 1964.

116. Smith, H. V. and J. M. K. Spalding. Outbreak of paralysis in Morocco due to ortho-cresyl phosphate. *Lancet* **2:** 1019–1021, 1959.

117. Sobue, I., K. Ando, M. Iida, T. Takayanagi, Y. Yamamura and Y. Matsuoka. Myeloneuropathy with abdominal disorders in Japan. A clinical study of 752 cases. *Neurology, Minneap.* **21:** 168–173, 1971.

118. Spencer, P. S., R. B. Hanna and G. D. Pappas. Acrylamide intoxication of Pacinian corpuscle function: a freeze fracture study. *J. Neuropathol. exp. Neurol.* **36:** 631, 1977 (abstract).

119. Spencer, P. S., R. Hanna, M. Sussman and G. Pappas. Inactivation of Pacinian corpuscle mechanosensitivity by acrylamide. *J. gen. Physiol.* **70:** 17a, 1977 (abstract).

120. Spillane, J. D. The geography of neurology. *Br. med. J.* **2:** 506–512, 1972.

121. Steiness, I. Vibratory perception in non-diabetic subjects during ischaemia with special reference to the conditions in hyperglycaemia, after carbohydrate starvation and after cortisone administration. *Acta med. scand.* **169:** 17–26, 1961.

122. Svennilson, E. Studies of triorthocresyl phosphate neuropathy, Morocco 1960. *Acta psychiat. scand.* Suppl. **150:** 334–336, 1960.

123. Swaiman, K. F. and D. Flagler. Mercury poisoning with central and peripheral nervous system involvement treated with penicillamine. *Pediatrics* **48:** 639–642, 1971.

124. Toole, J. F. and M. L. Parrish. Nitrofurantoin polyneuropathy. *Neurology, Minneap.* **23:** 554–559, 1973.

125. Towfighi, J., N. K. Gonatas, D. Pleasure, H. S. Cooper and L. McCree. Glue sniffer's neuropathy. *Neurology, Minneap.* **26:** 238–243, 1976.

126. Tsubaki, T., Y. Honma and M. Hoshi. Neurological syndrome associated with clioquinol. *Lancet* **1:** 696–697, 1971.

127. Tsubaki, T., K. Shirakawa, K. Hirota and K. Kanbayashi. Neurological aspects of methylmercury poisoning in Niigata. In: *Minamata Disease*, edited by T. Tsubaki and K. Irukayama. New York: Elsevier Publishing Company, 1977, pp. 145–165.

128. Tursky, B. and P. D. Watson. Controlled physical and subjective intensities of electric shock. *Psychophysiology* **1:** 151–162, 1964.

129. Ursing, B. and C. Kamme. Metronidazole for Crohn's disease. *Lancet* **1:** 775–777, 1975.

130. Urtasun, R. C., P. Band, J. D. Chapman, H. R. Rabin, A. F. Wilson and C. G. Fryer. Clinical phase I study of the hypoxic cell radiosensitizer Ro-07-0582, a 2-nitroimidazole derivative. *Radiology* **122:** 801–804, 1977.

131. Vasilescu, C. Sensory and motor conduction in chronic carbon disulphide poisoning. *Eur. Neurol.* **14:** 447–457, 1976.

132. Verrillo, R. T. Effect of contactor area on the vibrotactile threshold. *J. acoust. Soc. Am.* **35:** 1962–1966, 1963.

133. Verrillo, R. T. Vibrotactile thresholds for hairy skin. *J. exp. Psychol.* **72:** 47–50, 1966.

134. Vora, D. D., D. K. Dastur, B. M. Braganca, L. M. Parihar, C. G. S. Iyer, R. B. Fondekar and K. Prabhakaran. Toxic polyneuritis in Bombay due to ortho-cresyl-phosphate poisoning. *J. Neurol. Neurosurg. Psychiat.* **25:** 234–242, 1962.

135. Wahlberg, P., E. Karppanen, K. Henrikson and D. Nyman. Human exposure to mercury from goosander eggs containing methyl mercury. *Acta med. scand.* **189:** 235–239, 1971.

136. Whitelaw, D. M., D. H. Cowan, F. R. Cassidi and T. A. Patterson. Clinical experience with vincristine. *Cancer Chemother. Rep.* **30:** 13–20, 1963.

137. Wickersham, C. W. and E. J. Fredericks. Toxic polyneuropathy secondary to methyl n-butyl ketone. *Conn. Med.* **40:** 311–312, 1976.

138. Wong, P. P., A. Yagoda, V. E. Currie and C. W. Young. Phase II study of vindesine sulfate in the therapy for advanced renal carcinoma. *Cancer Chemother. Rep.* **61:** 1727–1729, 1977.

Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function
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Comparative Behavioral Toxicology[1]

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STEBBINS, W. C. AND D. B. MOODY. *Comparative behavioral toxicology.* NEUROBEHAV. TOXICOL. 1: Suppl. 1, 33–44, 1979.—Behavioral conditioning together with conventional sensory testing methods may be used in the evaluation of toxic effects on sensory systems in experimental animal models. Such procedures yield precise quantitative estimates of impairment in absolute and differential acuity and in sensory perception. Additionally, these behavioral changes can be related to the presence of histopathology in peripheral sensory structures; this orderly relation between structure and function may aid in our understanding of the basis for sensory coding in the normal end organ.

Behavioral toxicology Hearing loss Monkeys Guinea pigs Cats Chinchillas Drugs Noise

THE earliest behavioral indications of toxicity may often be subtle, and, in the absence of sufficiently sensitive measuring techniques, go undetected. The quest for methodology capable of detecting these early signs and, too, of determining the manner and precise action of toxic agents has led us to examine a variety of both methods and animal models. The comparative method is thus illustrated along two dimensions and will, we hope, add generality to our findings in order that we might more effectively extrapolate to man. It has been known for some time that certain drugs such as the aminoglycosidic antibiotics, and intense sound exposure can cause permanent deafness with severe cochlear histopathology in man. Our progress in achieving an understanding of the process and of the mechanisms in man himself is limited by our inability to control the relevant variables, such as the influence of other toxins, and to evaluate the histopathology immediately post mortem. It is for these reasons among others that we rely on animal models for their value in extrapolation to man.

First, in experiments on ototoxicity with the aminoglycosidic antibiotics and noise we have employed as subjects guinea pigs, chinchillas, cats, and several species of monkey. Thus, in a biological sense the approach can be considered comparative; in fact, we have discovered a striking example of species-specific toxicity in the course of this research.

Second, in the study of hearing and hearing loss brought about by these drugs and intense noise we have tried to utilize a variety of methods for our evaluation of impaired hearing. Hearing, like any other behavioral process, can be examined in more ways than one. The threshold test is perhaps the most widely used method of evaluation, and, in previous research, threshold changes have been related to histopathological changes occurring in the inner ear and central nervous system after exposure to toxic agents [8, 18, 23]. However, under normal lifelike conditions we seldom attend to stimulus events at minimum detectable levels. When hearing becomes impaired we undergo subtle changes in our ability to accurately locate and discriminate between acoustic events at normal speaking and listening levels. Consequently, it is important that we establish reliable and valid procedures for the evaluation of these suprathreshold discriminative functions.

This paper will consider some of the different characteristics of hearing and how they are measured in experimental animals before and after exposure to toxic agents. Examples will be drawn from different species and comparisons made between them. In most instances the similarity in toxic effects across species for a given agent is clear; however, there is at least one very interesting exception which, we think, illustrates the importance of diversity in both approach and choice of subject, particularly if our goal is to extend these findings to ourselves. Although our experience has been in the auditory system and the specific toxicants to which it is sensitive, there is every reason to believe that the behavioral methods described herein are applicable to the behavioral analysis of sensory impairment in general.

A significant outcome, even a gratuity, of these experiments on ototoxicity is that they have provided us with information about how the ear works. The relation between hearing loss and the changes occurring in the inner ear is a very predictable one. Stimulus coding of frequency and intensity is, in large measure, a function carried out by the receptor cells in the inner ear. These inner and outer hair cells are selectively destroyed by intense sound and certain of the ototoxic drugs with specific consequences for hearing, thus revealing certain key aspects of the peripheral coding process [21]. This issue will also be considered in this paper.

In behavioral, anatomical, and physiological studies of

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the auditory system, guinea pigs and cats have long been the subjects of choice. Although much of what we know about structure, function, and toxicity in the auditory system is based on findings from these animal models, they have often been considered recalcitrant subjects for behavioral experiments. However, we have found operant conditioning methods with the appropriate positive reinforcers to be quite effective [9, 10, 12]. In more recent years chinchillas [1,5] and monkeys [20] have been selected as subjects for auditory research both because of the relative ease with which they may be behaviorally trained for hearing testing and of the close semblance of their hearing thresholds to man's.

TRAINING AND TESTING PROCEDURES

We have employed the same basic training procedure with all of these animals. There are obvious and critical differences in some of the details such as the selection of the response device and the nature of the reinforcer, but a general training and threshold testing paradigm has been found effective for all. Further, only minor modifications in the basic paradigm for audiometric or threshold testing render it useful for a variety of discrimination procedures where the acoustic stimuli are well above threshold levels; i.e., frequency and intensity discrimination, loudness judgment, and so on [8].

Under the training procedure the animals are food deprived and food is then used as a reinforcer to strengthen desired behaviors. A stimulus light indicates the opportunity to respond by manually contacting either a switch or contact-sensitive plate. Contact, once initiated, must be maintained until an acoustic stimulus (pure tone) is presented. The time interval between contact initiation and stimulus onset is randomized on successive presentations. If manual contact is then broken while the tone is on, the food reinforcer is delivered immediately. The holding response is critical: if contact is broken prematurely, i.e., before the auditory stimulus is turned on, the trial is terminated without food and the next tone presentation (trial) may be delayed. Time intervals assigned are brief. The holding requirement can vary between 1 and 9 seconds. The penalty or time out for releasing the response device too soon may be a 6–10 sec delay before the stimulus light is turned on indicating the next trial. In addition, catch trials in which no stimulus is presented are interspersed randomly with tone trials in order to assess an animal's guess rate. In a more elaborate form of the procedure the trial is announced by a flashing light which becomes steady when the contact response has been effectively initiated. The light is turned off when food is given or if contact is prematurely broken.

Training begins with a conditioning session in which the animal is shaped by successive approximations to touch and maintain contact with the response device and continues until the contingencies described above are in effect and the discriminative behavior with regard to the auditory stimulus is stable [8]. Criteria for stability require that responding to catch trials is reduced to a low steady level and that the holding response is maintained until the tone is presented at which time contact is quickly broken. At this stage one of two psychophysical procedures for threshold testing is put into effect.

Under the constant stimulus method, tones at 5 intensities (equally spaced and bracketing the presumed threshold) are presented in a random order on successive trials. The frequency or percentage of times that the subject responds at each intensity level is noted and the threshold for a given frequency (pure tone) is that sound level to which the animal responds 50 percent of the time. A more efficient psychophysical method but also more difficult for the animal is that of tracking. The tone intensity is initially well above normal threshold. Each correct detection by the subject leads to a decrease in tone intensity on the subsequent trial. Failure to respond to the tone, conversely, is followed by an increase in tone intensity. Under this procedure the stimulus is quickly brought down by the subject to the threshold region and kept there (see Fig. 1). The difficulty lies in maintaining behavior which is continually under the control of barely audible stimuli. Threshold which is indicated by the dashed horizontal line in Fig. 1 is based on the average transition value between correct detections and failure to report the tone. Although the procedure has been used successfully with monkeys, the constant stimulus method appears more effective with guinea pigs. Thresholds are determined in this manner at frequencies encompassing an animal's entire audible range. Typical threshold functions are shown in Fig. 2 for four Old World monkeys (*Macaca*) and in Fig. 3 for two guinea pigs. These contours represent the minimum detectable sound pressure levels at various frequencies to which these animals can respond, and the functions for individual animals form baselines against which the effects of agents which damage the ear and impair hearing are measured. The data provide stable and reproducible measures of acoustic sensitivity in a variety of experimental animals; yet these measures are quick to reflect minute changes in the auditory system after insult with ototoxic compounds or exposure to intense sound.

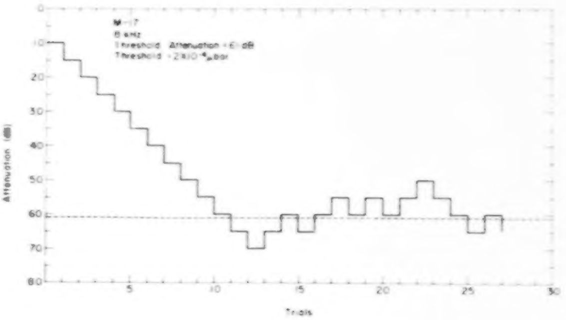

FIG. 1. Use of the tracking method for audiometric testing of monkeys. Correct detections cause the tone to be attenuated in 5-dB steps, while failure to hear produces a subsequent 5-dB increase in tone intensity. The threshold is indicated by the horizontal dashed line at 61 dB [18].

Absolute sensitivity is but one aspect of auditory function. An animal's survival and certainly man's ability to cope effectively with his environment depend on the discrimination of minimal differences in the waveform of acoustic stimulation. Such differential sensitivity may be evaluated by determining the smallest difference between two frequencies or two sound pressure levels to which an animal can respond. The testing procedure is very similar to that described above for the threshold test. The subject initiates a holding response which immediately produces a repetitively pulsed pure tone (standard frequency). After a brief but var-

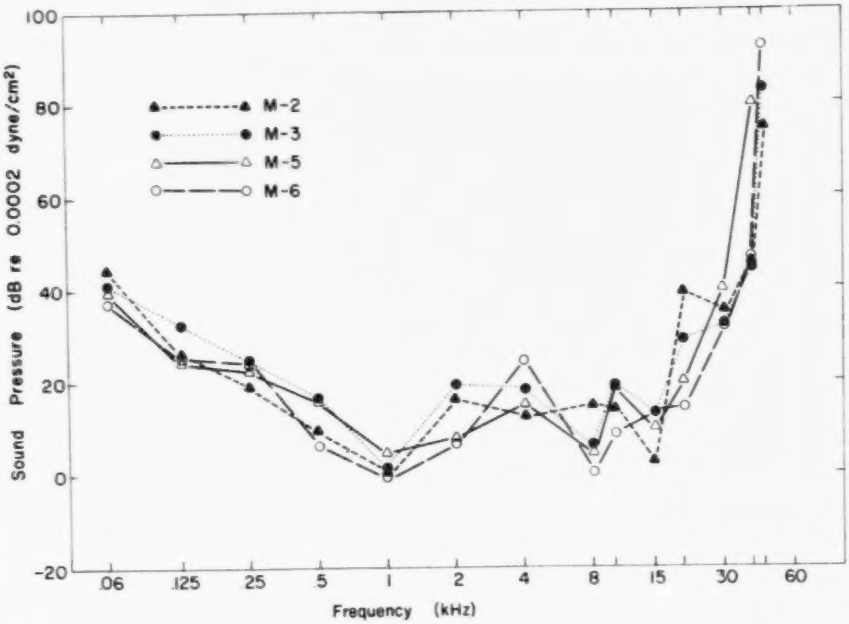

FIG. 2. Normal auditory thresholds for 4 macaque monkeys [15].

ied time interval, if the animal has maintained the contact response, a second pure tone (variable frequency) alternates several times with the first. The subject responds to the change in frequency by breaking contact and is then reinforced with food [8,16]. Following training the variable frequency is brought closer to the standard until the limits of the animal's resolution are reached and a differential threshold is determined as that frequency difference to which the subject responds 50% of the time. Differential thresholds for intensity or sound pressure may be determined with the same paradigm and are measured at sound pressure levels well above minimum detectable levels and, in the same manner as absolute threshold, across much of the ear's audible range. The significance of differential acuity for responding to the small nuances in the sounds of speech and communication, for example, can hardly be overestimated. The clinical audiometric examination includes similar tests in the diagnosis of hearing disorders.

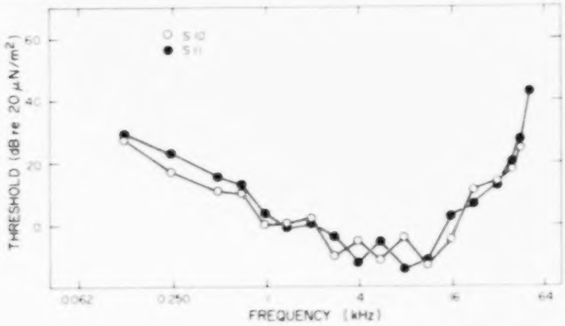

FIG. 3. Normal auditory thresholds for 2 guinea pigs [12].

Beyond the threshold measures of hearing capacity are the perceptual functions which assume importance at normal sound levels. One of these is the judgment of loudness. The absolute threshold function across frequency is the limiting case of the equal loudness contour. Significant decisions regarding, for example, the distance or exact location of a sound source are to a considerable extent based on loudness judgments. Recruitment refers to a form of hearing impairment in which our ability to discriminate loudness levels is markedly affected. In our laboratory we have devised a strategy for training experimental animals to make these judgments [6,14]. The paradigm is based on the fact that an animal's speed of reaction to the onset of an acoustic stimulus varies in an orderly fashion with the intensity of that stimulus [22]. A typical response latency-stimulus intensity function is shown in Fig. 4. Such differential responding at different stimulus levels provides us with a measure of loudness judgment and has been shown by us and others [11] to be quite compatible (in man) to loudness judgments made under more subjective (verbally instructed) conditions. Further, these latency-intensity functions give us an additional and different measure of auditory impairment. Not uncommonly we may find elevation of the hearing threshold but without significant hearing loss at typical conversational levels [7].

Training of animals to produce these loudness (latency-intensity) functions requires one simple elaboration on the basic threshold paradigm. Animals learn to report acoustic stimulation by breaking contact with a response device, but, in addition, they must do so quickly. In the course of training, food reinforcement is contingent upon a rapid response to tone onset. Such training ensures a relatively invariant response topography, response latencies on the order of 200 msec with minimal variability at high stimulus intensities.

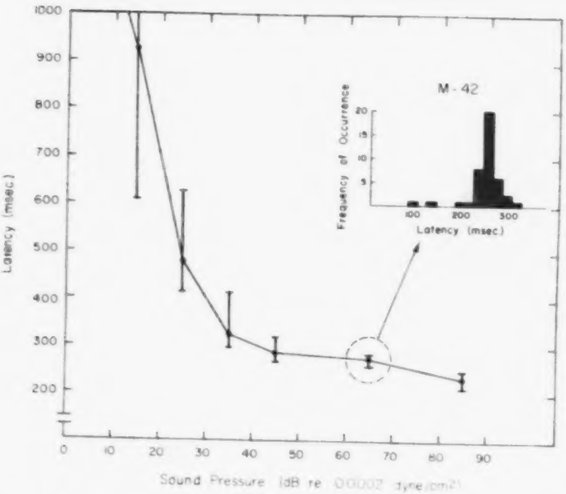

FIG. 4. Reaction time (medians as a function of stimulus intensity) for one macaque monkey. Vertical lines indicate semi-interquartile ranges. Inset, upper right, indicates frequency distribution of reaction times at 65 dB SPL from the reaction time-stimulus intensity function [17].

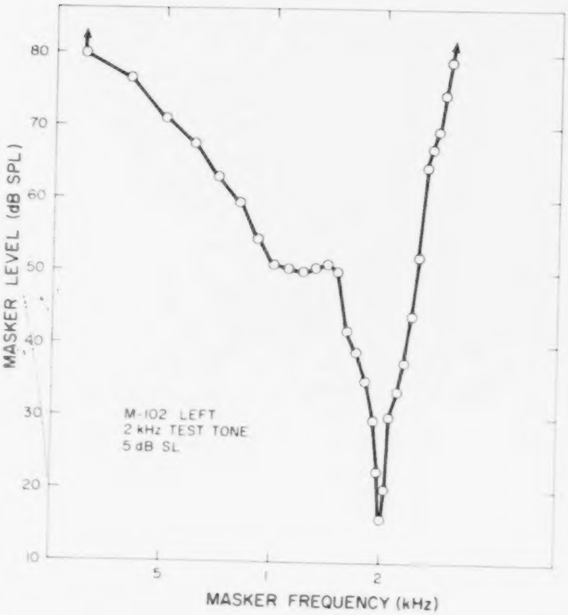

FIG. 5. Psychophysical tuning curve for a macaque monkey. The test tone was at 2 kHz, 5 dB above threshold.

and a latency-intensity function which covers most of the animal's dynamic range of hearing above absolute threshold.

There are yet other behavioral questions to be directed at the normally functioning and subsequently impaired auditory system. The acuity for the localization of sound in space is a binaural phenomenon which depends on the integrity of both ears and key portions of the central auditory system. Differential loss in one ear as, for example, with an eighth nerve tumor, leads to a deficiency in this important function. Behavioral measures of frequency analysis or selectivity, such as the critical band or psychophysical tuning curve, may provide an evaluation of inner ear function since the cochlea is the first and probably foremost frequency analyzer in the auditory system.

Tuning curves are well-known properties of single auditory neurons. More recently with behavioral techniques involving tone-on-tone masking we have been able to obtain psychophysical tuning curves in animals which closely resemble their electrophysiological counterparts [13]. One such function taken from a monkey is presented in Fig. 5. The test signal was a 2 kHz pure tone at 5 dB above threshold. The subject was conditioned to respond to the tone (as described above). Following correct detections the test tone remained at the same level but the intensity of the pure tone masker was raised. If the subject failed to report the test tone the intensity of the masker was decreased. The function in Fig. 5 represents the levels of various masker frequencies at which the 2 kHz test tone was just detectable. The discrimination measure of frequency selectivity is Q^{10dB} which is the test tone frequency divided by the bandwidth of the function 10 dB above the minimum. Human listeners with sensorineural hearing loss and probable cochlear involvement demonstrate pronounced changes in these functions suggesting a serious disruption of frequency analytic capabilities [4].

EXPOSURE TO OTOTOXIC AGENTS

We have been working principally with the aminoglycoside antibiotics kanamycin, neomycin, and dihydrostreptomycin in monkeys, guinea pigs, and cats or with intense sound exposure in monkeys and chinchillas. Drugs are administered daily following hearing testing. Treatment is continued until a given level of impairment has been produced. Animals are tested until their hearing loss has stabilized for at least one month following treatment when they are sacrificed for histologic evaluation. Sound exposures are given in eight-hour daily sessions followed by hearing testing. Animals are sacrificed one month or later after the final exposure.

HISTOLOGIC EVALUATION

At the time of sacrifice the animal's temporal bones are removed so that the cochlea of the inner ear may be prepared for examination and evaluation by phase contrast microscopy [2]. More detailed scrutiny may also be carried out with scanning or transmission electron microscopy. Under light microscopy inner and outer hair cells still present are counted and cytocochleograms, which display the number of hair cells as a function of their position on the basilar membrane, are constructed. Direct comparisons can then be made between these cochleograms and behavioral audiograms which represent the extent of the animal's hearing across the audible frequency range.

OTOTOXIC EFFECTS

Daily hearing testing both during and following administration of the toxicant reveals the progressive and orderly

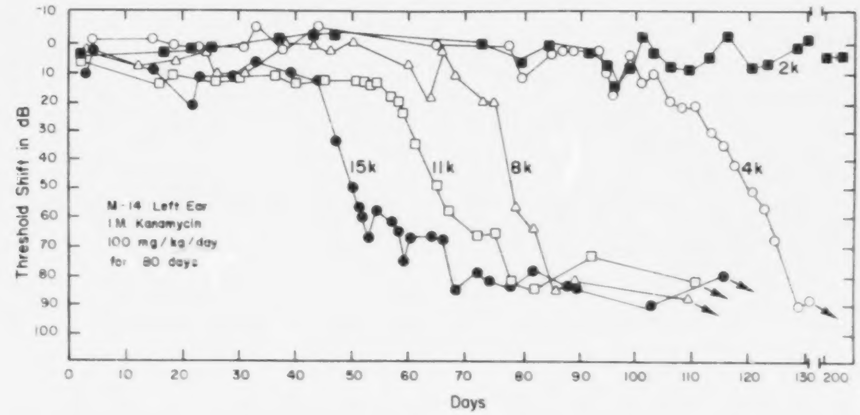

FIG. 6. Progressive changes in threshold for a macaque monkey for different acoustic frequencies during and following daily kanamycin treatment. The zero line represents normal hearing at all frequencies prior to drug treatment [23].

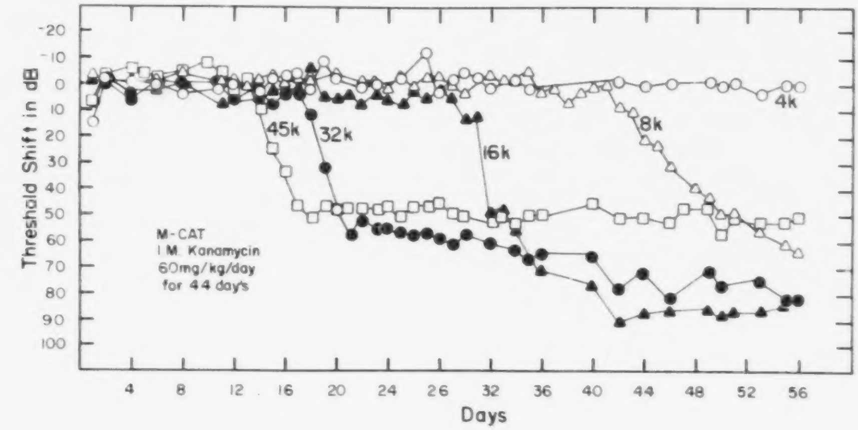

FIG. 7. Progressive changes in threshold for a cat for different acoustic frequencies during and following kanamycin treatment. The zero line represents normal hearing at all frequencies prior to drug treatment.

nature of the hearing loss. In Fig. 6 threshold changes at several frequencies are shown for a macaque monkey over a six-month period during which kanamycin was given daily. The results of hearing testing after cessation of treatment are also shown. Typically with kanamycin as with the other aminoglycosides hearing loss occurs first at the higher frequencies and inevitably spreads in time to the lows. If the antibiotic is continued, complete deafness ensues. The process resembles presbycusis (hearing loss with aging) although the time frame is considerably shorter. In Fig. 7 similar results are presented for a cat, and in Fig. 8 for a guinea pig. The similarities are striking. In addition to the gradual high-to-low frequency loss, the abruptness with which the loss occurs at each frequency is worth noting.

In Fig. 9 the terminal audiogram or threshold test (lower panel) for each ear before sacrifice is shown together with the cytocochleogram (upper panel) for right and left cochleas. The monkey is the same one described previously (Fig. 6). Note that while no response to the most intense acoustic stimuli could be obtained at the higher frequencies, hearing was completely normal at the lower frequencies. Our protocol requires testing until the degree of hearing loss has shown no further change for at least one month following the end of treatment; thus, there is every reason to consider the final thresholds as stable. The corresponding cytocochleogram is shown in the upper part of Fig. 9. While no inner or outer hair cells remain in the lower half of the cochlea, both kinds of cells are present and appear normal in the upper half. The symmetry of the pattern of missing hair cells and of the hearing loss in each ear is the usual finding in these animals. The boundary between present and missing hair cells at about 15 mm from the base resembles the sharp transition between normal hearing and complete deafness in the threshold function. The significance of this relation be-

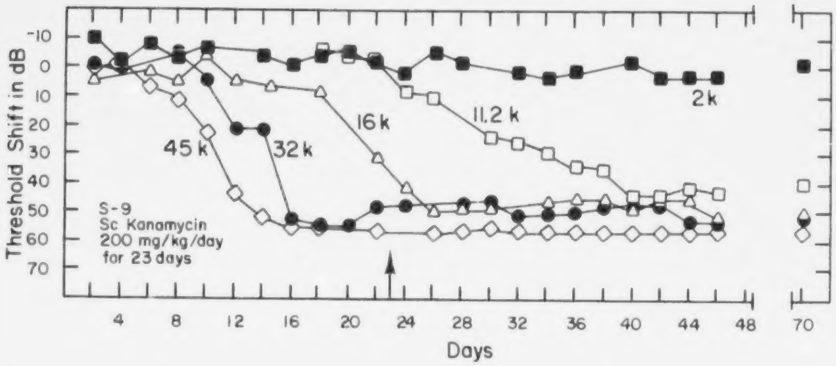

FIG. 8. Progressive changes in threshold for a guinea pig for different acoustic frequencies during and following daily kanamycin treatment. The zero line represents normal hearing at all frequencies prior to drug treatment.

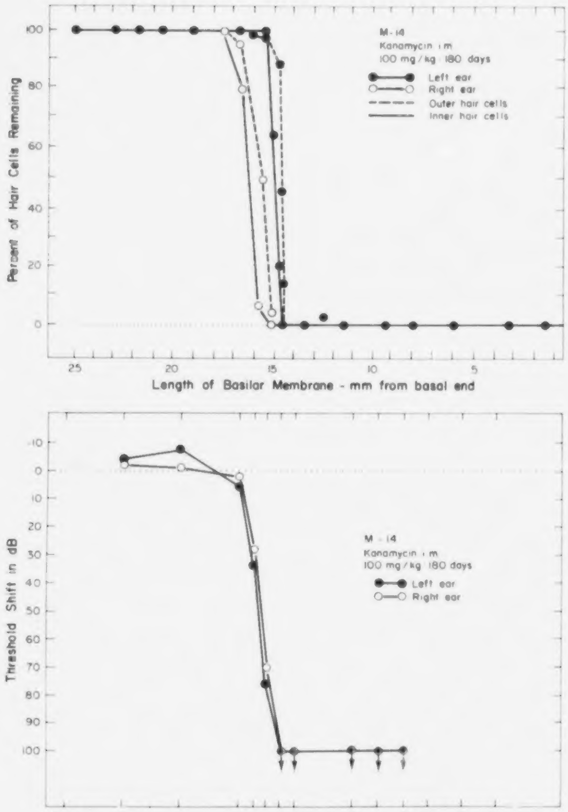

FIG. 9. Auditory threshold shift for the monkey represented in Fig. 6 after 180 days of daily kanamycin (lower panel); hair cells remaining as a function of position on basilar membrane (upper panel) [19].

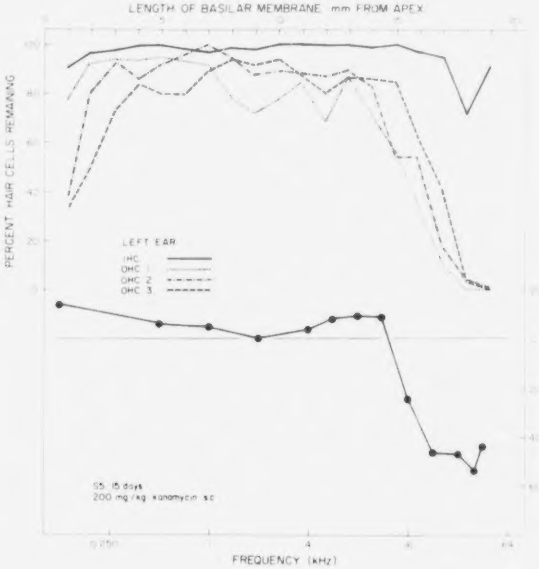

FIG. 10. Auditory threshold shift (lower panel) for a guinea pig measured 5 weeks after last treatment with kanamycin and cytocochleogram (upper panel) [12].

tween hearing impairment and loss of receptor cells in the inner ear for stimulus frequency coding in the auditory periphery will be discussed later.

In Figs. 10 and 11 the final audiograms taken prior to sacrifice are shown together with the cytocochleograms for two kanamycin-treated guinea pigs. The animal whose data are presented in Fig. 11 is the same one whose progressive loss was shown in Fig. 8. The familiar pattern of severe high-frequency hearing loss with normal hearing at the low frequencies can be observed. Two important differences between these results for the guinea pigs and those for the monkey should be noted. First, the guinea pigs are not as severely deafened at the higher frequencies but retain some measurable hearing. Second, there is clear evidence of

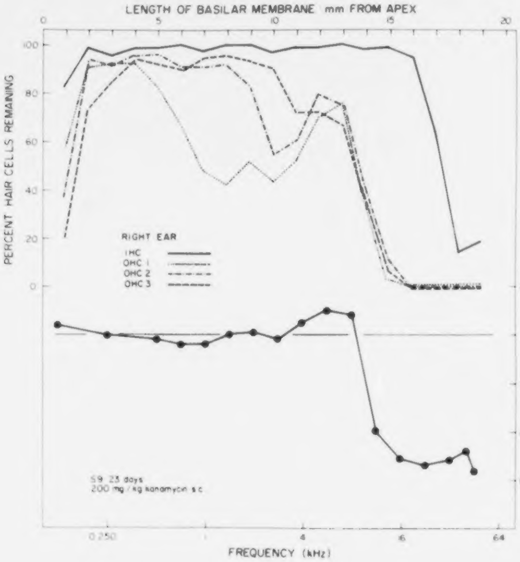

FIG. 11. Auditory threshold shift (lower panel) for the guinea pig represented in Fig. 8 measured 5 weeks after last treatment with kanamycin and cytocochleogram (upper panel) [12].

differential receptor cell loss in the cochleas of the guinea pigs. Many more outer than inner hair cells are missing from the basal region of the cochlea. The possible significance of these findings for stimulus intensity coding will be discussed below.

One of our most interesting and serendipitous discoveries was the finding of a form of species-specific toxicity in nonhuman primates [3]. This is a finding with clear implications for the use of animal models in toxicologic research and one which should be pursued. Macaque monkeys appear relatively insensitive (with regard to their hearing) to dihydrostreptomycin at dose levels up to 100 mg/kg (5 times the clinical dose in man) for daily injection periods of as long as eight months. Yet patas monkeys suffer considerable irreversible hearing impairment in a matter of weeks when given the clinical dose. Examples with thresholds taken one week after cessation of treatment and again when hearing loss had stabilized just before sacrifice together with cytocochleograms are shown in Figs. 12 and 13.

These data are very similar to those just described for the kanamycin-treated guinea pigs. The hearing loss progressed from the high frequency end of the audible range toward the lows. Failure to hear at all at the highest frequencies tested, a moderate to severe loss over the midrange, and normal or nearly normal hearing at the lowest frequencies characterize these monkeys. Correlated with the behavioral impairment was a complete loss of all receptor cells in the extreme base of the cochlea, a long stretch of membrane with normal appearing inner hair cells and no outer hair cells, and finally a full or nearly full complement of both cell types in the apex of the cochlea. While these effects were observed in the patas monkeys, hearing in the treated macaques remained normal and there were no significant alterations in their cochlear morphology. Seven patas and six macaques were examined in this experiment. With regard to sensitivity to

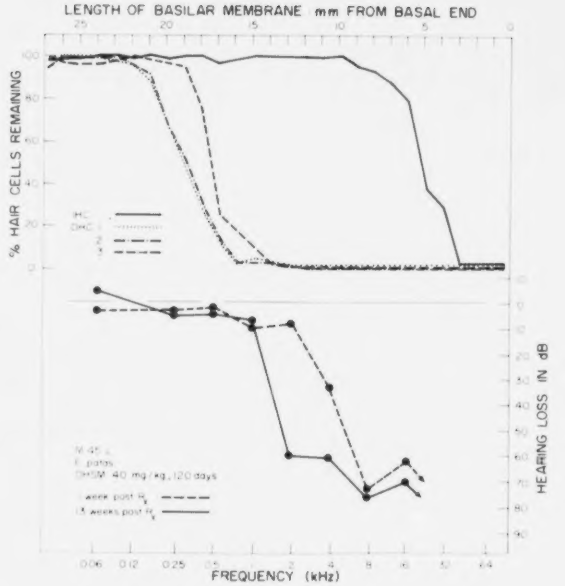

FIG. 12. Auditory threshold shift (lower panel) and cytocochleogram (upper panel) for a patas monkey treated with dihydrostreptomycin. The dashed function represents hearing loss 1 week after drug treatment was stopped; the solid function indicates hearing loss 12 weeks later [3].

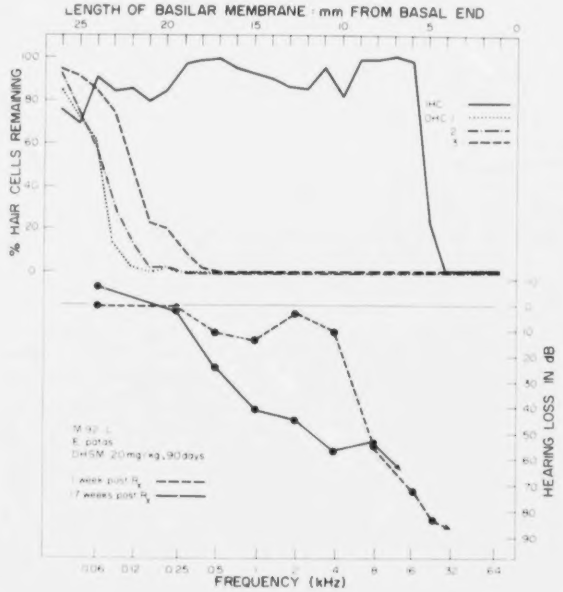

FIG. 13. Auditory threshold shift (lower panel) and cytocochleogram (upper panel) for a patas monkey treated with dihydrostreptomycin. The dashed function represents hearing loss 1 week after drug treatment was stopped; the solid function indicates hearing loss 17 weeks later [3].

dihydrostreptomycin, man is probably somewhere between these two extremes: a small but significant number of humans treated with dihydrostreptomycin experience severe hearing loss. A unique part of the effect for this drug in both man and patas is its delayed toxic action (see Figs. 12 and 13). Months following the last treatment, the patas monkeys continued to experience a deterioration in their hearing. We rarely see this degree of delayed effect with the other antibiotics. The continued daily testing protocol brings it out very clearly.

Though we tend to consider as toxicants only those liquids, gases or solids which threaten our health or even our life, intense sound as a severe and usually transitory mechanical disturbance in air should qualify as a health-threatening pollutant, and therefore the appropriate subject matter of toxicology. Although different from other toxicants in that no lingering residue remains in tissue or in the environment, nonetheless intense sound leaves its permanent mark at least on hearing and on the inner ear and undoubtedly on psychological functions less subject to precise measurement. Unlike the antibiotics, intense sound or noise can cause temporary hearing loss but depending on its intensity and duration it can also produce permanent loss in the same manner as the antibiotics by destroying the receptor cells in the inner ear. However, the pattern of destruction is often somewhat different and depends to some extent on the spectral properties of the sound source.

Figure 14 illustrates an example of noise-induced permanent hearing loss in a chinchilla exposed to band limited noise (710 to 2800 Hz) at 123 dB SPL for only 15 min. A moderate to severe loss is evident throughout the animal's hearing range (lower panel). Outer hair cells have disappeared throughout the middle and basal regions of the cochlea, while considerably more than 50% of the inners remain. The final audiogram was taken one month after the noise exposure. In Fig. 15 we show the results for the left ear of a monkey exposed on a working day schedule for three months to the intense and complex noise recorded from an automotive stamping plant and played back at the same level (105 dBA with impulse peaks reaching 126 dB SPL). The hearing loss though moderate is significant (see lower panel) and is correlated with a pronounced loss of first row outer hair cells. The differential sensitivity of inner and outer hair cells to ototraumatic insult, whether by drugs or intense sound, is readily apparent.

Measures of absolute threshold or the minimum detectable levels of acoustic energy by the ear provide an evaluation of one important aspect of auditory function. Agents, toxic at least to the peripheral auditory system, destroy the receptor cells which lie along the basilar membrane of the cochlea of the inner ear, and these events are followed by loss of cochlear supporting cells and degeneration of the auditory nerve and even cellular destruction at the level of the cochlear nucleus in the brain stem. Behavioral threshold testing either at high frequencies after antibiotic therapy or at those frequencies which are most intense in a high-level exposure sound or noise provide the earliest evidence of the beginning of the toxic process. We consider this kind of early warning system to be a most important goal for behavioral toxicology.

Differential sensitivity to frequency or other dimensions of acoustic stimulation may tell us something about an animal's ability to resolve those dimensions. In Fig. 16 the standard audiogram is plotted on the right for an animal treated with kanamycin. The familiar shape of the post drug function

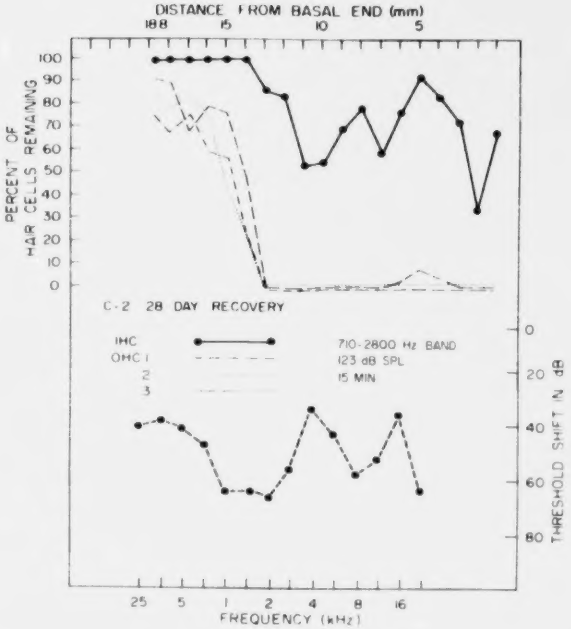

FIG. 14. Auditory threshold shift for a chinchilla after exposure to band-limited noise (lower panel) and cytocochleogram (upper panel) [1].

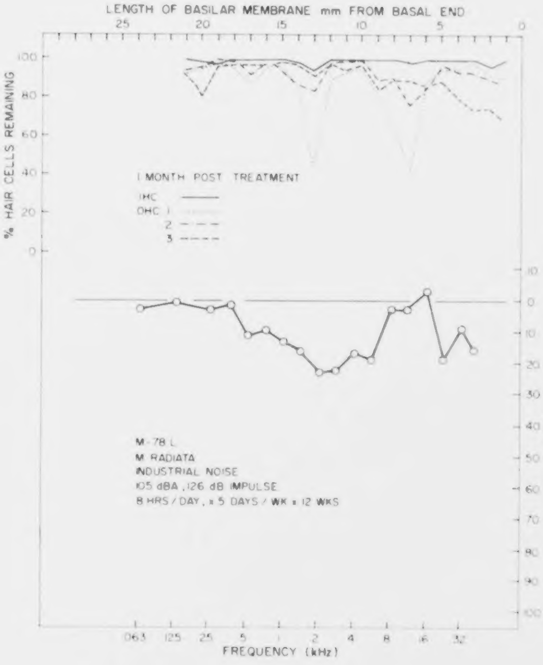

FIG. 15. Auditory threshold shift (lower panel) and cytocochleogram (upper panel) for a macaque monkey exposed to industrial noise.

needs no further comment. The animal was trained and tested on alternate days for his ability to discriminate changes in frequency—the frequency difference threshold. The data indicate that impairment in frequency discrimination precedes the shifts in absolute threshold. Although the animal's ability to hear frequencies up to 4 kHz is normal, he is significantly impaired at higher frequencies. Yet at 4 kHz differential sensitivity to frequency is significantly affected. Additional measures such as this provide alternate ways of examining the effects of ototoxins.

The difficulties in training an animal on two different tasks is outweighed by the information gained. For example, in Fig. 17 audiometric (threshold) findings are presented for a patas monkey treated with dihydrosteptomycin. After 75 days of treatment a moderate high frequency loss was observed. For three months following treatment the hearing loss progressed to the low frequencies. If we now examine the animal's reaction time—stimulus intensity functions at a single key frequency (8 kHz), the progressive nature of the post-drug hearing loss becomes apparent (Fig. 18). The greatest loss occurred in the first two weeks following treatment, and particularly significant is the observation that the loss was more severe at intensity levels near threshold. Note that the functions come together at high sound levels suggesting little change in sensitivity at these levels. The effect, commonly noted with sensorineural hearing loss in man, is referred to as loudness recruitment. The reaction time procedure affords us an opportunity to study auditory perception in experimental animal models and its deterioration after exposure to toxic substances. It also gives us an added insight into the detailed behavioral changes occurring after such exposure.

A somewhat similar pattern of loudness recruitment is seen in Fig. 19 for a monkey following exposure to an octave band of noise centered at 2 kHz. Recruitment tests were carried out at 500, 2000, 4000, and 8000 Hz. Significant effects on hearing are seen only at 2000 and 4000 Hz, and, similar to the drug-treated animal described above, these effects are obvious at low stimulus levels near absolute threshold. The effects of the same exposure level and duration are more pronounced for a second animal whose recruitment functions are shown in Fig. 20. Sizeable shifts in the latency-intensity functions occurred at all frequencies close to and above the noise exposure band. This animal's separately determined threshold function measured over the same four test frequencies together with the cytocochleogram is presented in Fig. 21. As seen before, significant high frequency hearing loss is correlated with complete loss of outer hair cells and some sporadic loss of inners in the basal half of the cochlea. The recruitment functions indicate a sizeable impairment in loudness discrimination near threshold and at moderate sound levels perhaps 40 to 50 dB above threshold; at higher levels still, hearing is relatively normal although the animal's dynamic (intensity) hearing range is considerably compressed.

SENSORY CODING IN THE EAR

The orderly and predictable nature of the deterioration in hearing and the related disappearance of the receptor cells in the inner ear following toxic insult may tell us something of the function of these cells in normal hearing. The conceptual notion of frequency coding according to place of stimulation along the basilar membrane in the cochlea from base to apex is widely held. If we consider other data similar to those shown in Fig. 9 we can begin to generate an actual frequency map of the cochlear spiral as presented in Fig. 22 based on data from four drug-treated animals. The frequency scale for threshold testing is aligned with the linear extent of the basilar membrane according to certain rules which are consistent between animals. The result is strongly supportive of a place principle of frequency coding and yields a frequency map of the monkey's cochlea.

Intensity coding in the mammalian inner ear is still poorly understood. Some coding is surely done in single auditory nerve fibers but this seems insufficient to account for the enormous (greater than 100 dB) dynamic range of the mammalian ear. One reasonable possibility depends on a functional difference between the inner and outer hair cells not dissimilar to the differences observed for the rods and cones of the vertebrate retina. The receptor cells in the ear may be part of a two stage intensity code with the more delicate outer hair cells subserving sound pressure levels from threshold to about 50 dB above threshold and the inner hair

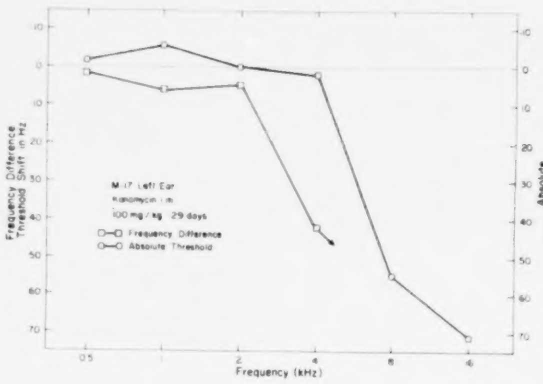

FIG. 16. Changes in absolute auditory threshold and frequency difference threshold in a macaque monkey treated with kanamycin [18].

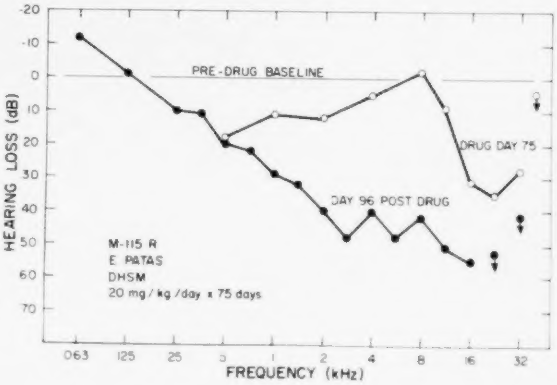

FIG. 17. Auditory threshold shift for a patas monkey treated with dihydrostreptomycin. The upper function represents the threshold shift at the end of drug treatment; the lower function was taken more than 3 months following the end of drug treatment.

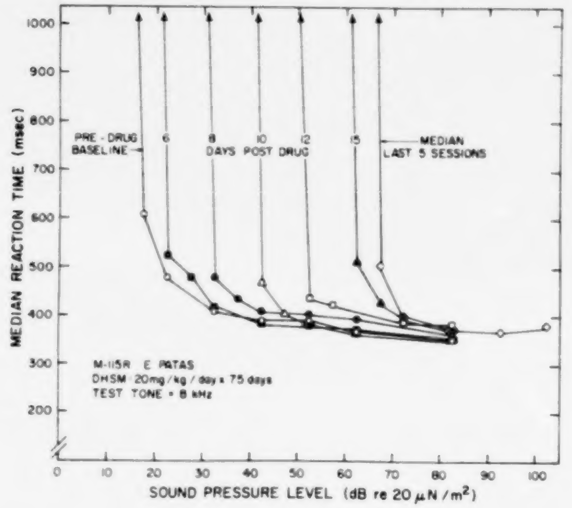

FIG. 18. Reaction time-stimulus intensity functions at 8 kHz for the same monkey shown in Fig. 17 before and varying times after drug treatment.

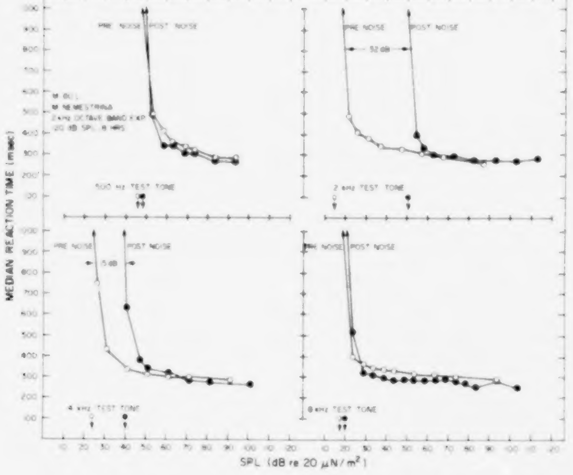

FIG. 19. Reaction time-stimulus intensity functions for a macaque monkey at 4 test tone frequencies before and after exposure to noise. Arrows near abscissa indicate pre- and post-exposure thresholds at those test tone frequencies.

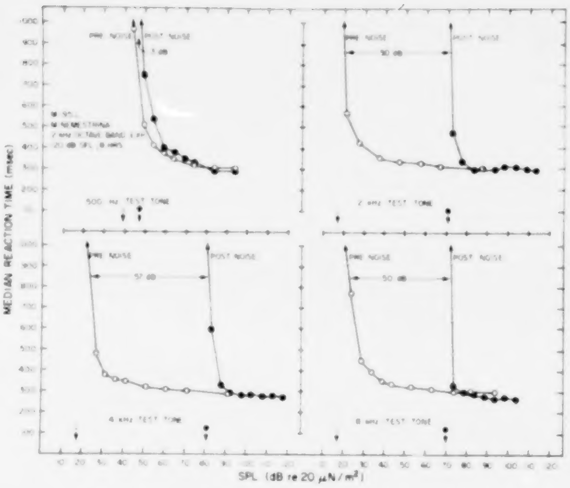

FIG. 20. Reaction time-stimulus intensity functions for a macaque monkey at 4 test tone frequencies before and after exposure to noise. Arrows near abscissa indicate pre- and post-exposure thresholds at those test tone frequencies.

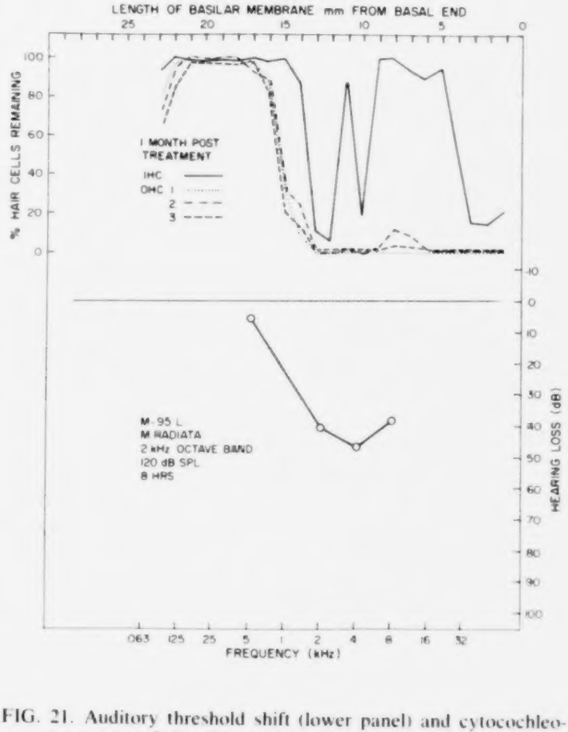

FIG. 21. Auditory threshold shift (lower panel) and cytocochleogram (upper panel) for the monkey whose reaction time data are shown in Fig. 20.

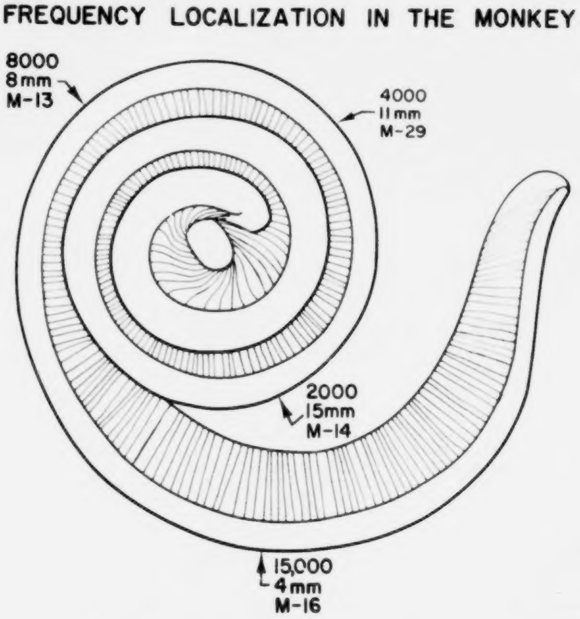

FIG. 22. Cochlear locations of the regions of threshold responses at 15, 8, 4 and 2 kHz in the monkey as determined in experiments with kanamycin and neomycin [18].

cells taking over from 50 dB to the higher sound levels. Our data form only one line of evidence in support of this notion. Note for example in Figs. 10–13 in those basal portions of the cochlea, where only inner hair cells remain, there is about a 50 dB hearing loss at the frequencies corresponding to those locations on the basilar membrane. We suggest that the inner hair cells are viable and therefore responsible for the residual hearing in those ears. If the inner hair cells are also removed as in Fig. 9 all traces of hearing disappear and the impairment is complete. An intermediate example with hearing loss at some frequencies greater than 50 dB accompanied by inner hair cell loss is seen in Fig. 14.

SUMMARY

Behavioral methods for the evaluation of the effects of toxins on hearing in experimental animal models have been described. Measures of threshold and differential sensitivity, of frequency selectivity, and of loudness discrimination were discussed. Representative findings from guinea pig, chinchilla, cat, and monkey were shown following treatment with one of the aminoglycosidic antibiotics or exposure to intense sound. The behavioral methods should not be considered limited to hearing testing for they have more general application in the assessment of toxic effects on sensory systems in a wide variety of mammalian species. Further, experiments such as those described herein may yield significant information concerning the basic mechanisms underlying sensory processing in the unimpaired or normal animal.

REFERENCES

1. Clark, W. W., C. L. Clark, D. B. Moody and W. C. Stebbins. Noise-induced hearing loss in the chinchilla determined by a positive reinforcement technique. *J. Acoust. Soc. Am.* **56:** 1202–1209, 1974.
2. Hawkins, J. E., Jr. and L.-G. Johnsson. Microdissection and surface preparations of the inner ear. In: *Handbook of Auditory and Vestibular Research Methods*, edited by C. Smith and J. Vernon. Springfield, Ill.: Charles C. Thomas, 1976, pp. 5–52.
3. Hawkins, J. E., Jr., W. C. Stebbins, L.-G. Johnsson, D. B. Moody and A. Muraski. The patas monkey as a model for dihydrostreptomycin ototoxicity. *Acta. Otolaryngol.* **83:** 123–129, 1977.
4. Hoekstra, A. and R. J. Ritsma. Perceptive hearing loss and frequency selectivity. In: *Psychophysics and Physiology of Hearing*, edited by E. F. Evans and J. P. Wilson. New York: Academic Press, 1977, pp. 263–271.
5. Miller, J. D. Audibility curve of the chinchilla. *J. Acoust. Soc. Am.* **48:** 513–523, 1970.
6. Moody, D. B. Reaction time as an index of sensory function. In: *Animal Psychophysics: The Design and Conduct of Sensory Experiments*, edited by W. C. Stebbins. New York: Appleton-Century-Crofts, 1970, pp. 277–303.
7. Moody, D. B. Behavioral studies of noise-induced hearing loss in primates: loudness recruitment. In: *Advances in Otorhinolaryngology: Otophysiology*, edited by J. E. Hawkins, Jr. and M. Lawrence. Basel: Karger, 1973, pp. 82–101.
8. Moody, D. B., M. D. Beecher and W. C. Stebbins. Behavioral methods in auditory research. In: *Handbook of Auditory and Vestibular Research Methods*, edited by C. Smith and J. Vernon. Springfield, Ill.: Charles C. Thomas, 1976, pp. 439–497.
9. Orr, J. L., D. B. Moody and W. C. Stebbins. Behavioral system and apparatus for tone detection and choice reaction times in the cat. *J. Acoust. Soc. Am.* **62:** 1268–1272, 1977.
10. Petersen, M. R., C. A. Prosen, D. B. Moody and W. C. Stebbins. Operant conditioning in the guinea pig. *J. exp. Analysis Behav.* **27:** 529–532, 1977.
11. Pfingst, B. E., R. Hienz, J. Kimm and J. Miller. Reaction-time procedures for measurement of hearing. I. Suprathreshold functions. *J. Acoust. Soc. Am.* **57:** 421–430, 1975.
12. Prosen, C. A., M. R. Petersen, D. B. Moody and W. C. Stebbins. Auditory thresholds and kanamycin-induced hearing loss in the guinea pig assessed by positive reinforcement procedures. *J. Acoust. Soc. Am.* **63:** 559–566, 1978.
13. Serafin, J. V., D. B. Moody and W. C. Stebbins. Psychophysical tuning curves in monkeys. *J. Acoust. Soc. Am.* **63:** S30, 1978.
14. Stebbins, W. C. Auditory reaction time and the derivation of equal loudness contours for the monkey. *J. exp. Analysis Behav.* **9:** 135–142, 1966.
15. Stebbins, W. C. Studies of hearing and hearing loss in the monkey. In: *Animal Psychophysics: The Design and Conduct of Sensory Experiments*, edited by W. C. Stebbins. New York: Appleton-Century-Crofts, 1970, pp. 41–66.
16. Stebbins, W. C. Hearing of Old World monkeys *(Cercopithecinae). Am. J. Phys. Anthro.* **38:** 357–364, 1973.
17. Stebbins, W. C., C. H. Brown and M. R. Petersen. Sensory processes in animals. In: *Handbook of Physiology, Sensory Processes, Vol. I*, edited by I. Darian-Smith, J. Brookhart and V. B. Mountcastle, section eds. Washington, D.C.: American Physiological Society, 1979, in press.
18. Stebbins, W. C., W. W. Clark, R. D. Pearson and N. G. Weiland. Noise- and drug-induced hearing loss in monkeys. In: *Advances in Otorhinolaryngology: Otophysiology*, edited by J. E. Hawkins, Jr. and M. Lawrence. Basel: Karger, 1973, pp. 42–63.
19. Stebbins, W. C. and S. Coombs. Behavioral assessment of ototoxicity in nonhuman primates. In: *Behavioral Toxicology*. New York: Plenum Press, 1976, pp. 401–427.

20. Stebbins, W. C., S. Green and F. L. Miller. Auditory sensitivity of the monkey. *Science* **153:** 1646–1647, 1966.
21. Stebbins, W. C., J. E. Hawkins, Jr., L.-G. Johnsson and D. B. Moody. Hearing thresholds with outer and inner hair cell loss. *Am. J. Otolaryngol.*, 1979, in press.
22. Stebbins, W. C. and J. M. Miller. Reaction time as a function of stimulus intensity for the monkey. *J. exp. Analysis Behav.* **7:** 309–312, 1964.
23. Stebbins, W. C., J. M. Miller, L.-G. Johnsson and J. E. Hawkins, Jr. Ototoxic hearing loss and cochlear pathology in the monkey. *Trans. Am. Otol. Soc.* **57:** 110–128, 1969.

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Trialwise Tracking Method for Measuring Drug-Affected Sensory Threshold Changes in Animals[1]

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ANDO, K. AND K. TAKADA. *Trialwise tracking method for measuring drug-affected sensory threshold changes in animals.* NEUROBEHAV. TOXICOL. **1:** Sappl. 1, 45–52, 1979.—Rats and rhesus monkeys were trained under a multiple schedule, the components of which were random ratio schedules for food presentation and for shock presentation. The discriminative stimulus for the shock presentation component was a pure tone for the rats and a light for the rhesus monkeys. In the test session under the extinction condition for the shock presentation component, the intensity of the discriminative stimulus was successively either decreased by fixed units when the conditioned suppression was observed or increased when the conditioned suppression was not observed. The levels finally oscillated within a narrow range around the threshold. The auditory thresholds of rats were increased by intramuscular administration of quinidine at 20 mg kg and also by repeated intramuscular administration of kanamycin at 250 and 500 mg kg day. In rhesus monkeys, visual thresholds were raised by application of pilocarpine at 0.02–0.16 mg kg to the eyes and also by subcutaneous administration of LSD-25 at 4–8 μg kg in one monkey and at 20–30 μg kg in another. The method used for tracking the animals' sensory thresholds was sensitive enough to test the selective effect of the drugs and was also a relatively easy way to obtain a stable behavioral baseline for experimental purposes.

Trialwise tracking method Auditory threshold Quinidine Kanamycin Rat Visual threshold
Pilocarpine LSD-25 Rhesus monkeys

ACCORDING to clinical reports, a number of drugs have been found to affect sensory functions. For example, aminoglycoside antibiotics (e.g., kanamycin), diuretics (e.g., ethacrynic acid), salicylates, quinidine and quinine, etc., have toxic effects on auditory function while cholinomimetic drugs (e.g., pilocarpine), LSD-25, chloroquine (an antimalarial drug), ethanbutol (an antitubercular drug), etc., have various effects on visual function [10,15].

It is important to know whether such effects on sensory functions as have been observed in man can be reproduced in animal experiments. One must determine whether sensory toxic effects similar to those observed in clinical situations may be obtained in animal experiments, whether effects are specific to a certain sensory function and are reversible, what relationship exists between the effect and the conditions of administration (e.g., dose, route, whether single or repeated administration), and what the mechanism of the action is. To answer these questions, there is an urgent need to establish appropriate behavioral as well as physiological and histopathological methodologies. A variety of behavioral methods have been used in this area. Some examples are the unconditioned pinna reflex elicited by tonal stimulus in guinea pigs [1], the pole climbing response in rats [8], and conditioned inhibition of cold shivering in refrigerated guinea pigs [2,9], all of which have been used to test the ototoxic effects of antibiotics. Operant procedures such as multiple schedules [12, 20, 21], choice situations [4,23], an avoidance situation [18] and a conditioned suppression paradigm [6] have also been used to test drug effects on sensory functions. However, these methods do not directly test the drug effects on sensory thresholds. In contrast, there exists a more sophisticated operant approach called the "tracking" method (also known as the "stair case," "up and down" or "titration" method) which does test drug-affected sensory threshold changes directly. In this method, the stimulus presentation is essentially response-dependent. For example, one type of response leads to increments in the intensity of a stimulus while another leads to decrements, and the consequence is that the increments and decrements gradually tend to oscillate within a narrow range about the threshold value

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[5,24]. In the application of this type of method in drug studies, increased auditory threshold changes have been successfully observed after repeated administration of kanamycin in monkeys [26,27], and in rats [16], and increased visual threshold changes have also been observed after single administration of LSD-25 in pigeons [3] and after repeated administration of trans 11-amino-10, 11-dihydro-5-(3-dimethylaminopropyl)-5, 10-epoxy-5H-dibenzo[a,d]-cycloheptene dihydrochloride in dogs [17]. Although fewer trials are required with the tracking method because most stimuli are presented near the threshold level, it usually requires a long training period as well as a complex apparatus [5]. However, there have been some studies in which sensory thresholds were tested in animals using tracking methods adopting a conditioned suppression paradigm [16, 19, 22, 24, 25, 28]. In this so-called trialwise tracking approach [5], the easy establishment and very effective stimulus control of the conditioned suppression provide a considerable advantage.

The purpose of the present study was to test the effects of quinidine and kanamycin on the auditory threshold in rats and also the effects of pilocarpine and LSD-25 on the visual threshold in rhesus monkeys by using a trialwise tracking method adopting a conditioned suppression paradigm.

EXPERIMENT 1

The effect of a single administration of quinidine and of repeated administration of kanamycin on auditory threshold were tested in rats using a trialwise tracking method.

METHOD

Animals

Six experimentally naive, adult male Wistar rats were used. They were maintained at approximately 80% of their free-feeding body weights throughout the experiment.

Apparatus

Two operant-conditioning chambers (Lehigh Valley Electronics Inc., Model 143-22) mounted in sound-attenuating cubicles (40×112×40 cm) were used. Each chamber had a lever which was located 6 cm above the floor and 7.5 cm to the right of the vertical midline of the front wall. A minimum force of 20 g was required to operate the lever. A food pellet weighing 45 mg was dispensed into a hopper from a dispenser (Lehigh Valley Electronics Inc., Model 114-20) located behind the front wall of the chamber. An interrupted pure tone (10 KHz) used as the test stimulus was generated by an oscillator (Nagashima Medical Instruments Co., Model PA) through a speaker (Foster Electric Co., Model UP-163). The speaker was located 60 cm away from the front wall towards the rear. The rear wall was made of wire mesh to allow the tone to pass through freely. The tone was presented for 1 sec intervals separated by 1 sec intervals of silence. The intensity of the tone was adjusted manually by turning the oscillator dial, which had been calibrated by a sound level meter (Brüel and Kjäer Co., Model 2107) attached to a condenser microphone (Brüel and Kjäer Co., Model 4144) which was located in the middle of the chamber and was pointed toward the speaker. A green lamp (dia. 1.2 cm, 24 VDC) amounted 4 cm above the lever was used as a control discriminative stimulus (the control light). Two mA of electric shock were delivered to the grid floor of the chamber via a shocker. A fluorescent lamp attached to the sound-attenuating cubicle provided continuous illumination in the chamber. Experimental contingencies and data recording were arranged by a PDP 8/I computer (Digital Equipment Co.). Cumulative response recorders (Ralph Gerbrands Co., Model C-3) were also used. This controlling and recording equipment was located in the adjacent room.

Procedure

Training under the multiple schedule. Rats were trained to press a lever for a food pellet under a random ratio schedule in which lever pressing was reinforced randomly on the average of every 20 presses (RR 20 schedule). After responding by the rats stabilized under the RR 20 schedule for food reinforcement, training under a multiple schedule [13] was begun. A discriminative stimulus, either the pure tone (frequency, 10 KHz; sound pressure level, 60 dB re 0.0002 dyne/cm^2) or the control light was presented for 1 min every 10 reinforcements while the rats were responding for food under the RR 20 schedule. During presentation of the discriminative stimulus, responding was shocked under the RR 20 schedule instead of delivery of a food pellet (mult RR 20 [food] RR 20 [shock] schedule). One training session under the multiple schedule included 10 to 15 food presentation periods (food periods) and the same number of discriminative stimulus presentation periods with shock (stimulus w/shock periods) occurring alternately and beginning with a food period. Two of the stimulus w/shock periods were chosen randomly to be governed by the control light and all the other stimulus w/shock periods occurred in conjunction with the tone. This multiple schedule training was held every day except Sunday.

Threshold test. The auditory threshold test sessions were given after a stable high rate of responding during the food periods and suppression of responding in the stimulus w/shock periods were observed. These test sessions were generally given once a week avoiding Monday, the rest of the week being occupied by training sessions under the multiple schedule. The procedure for the test sessions was the same as for training sessions except that responding by the rat during presentation of the discriminative stimulus was not shocked (stimulus periods), and the intensity of the test tone in each stimulus period was changed according to the degree of conditioned suppression recorded during each preceeding tone presentation. The degree of the conditioned suppression was expressed by the suppression ratio (SR), defined as follows:

$$SR = \frac{B-A}{B}$$

A: Response rate during the last stimulus period.
B: Response rate during the food period between the last two stimulus periods.

The sound pressure of the tone for each stimulus period was decreased by 4, 6, or 10 dB steps when the SR was greater than or equal to 0.90 and was increased by 4, 6, or 10 dB steps when the SR was less than 0.90. The levels finally oscillated within a narrow range around the threshold. The intensity of the control light was kept constant in Experiment 1.

Drug tests. After consistent and reproducible tracking data were obtained in the threshold test, the drug test sessions were conducted in the same manner as the threshold test sessions. In the drug test involving quinidine using 2 rats, vehicle only, carboxymethylcellulose in saline at 0.5%, was administered at 1 ml/kg IM, 95 min before the first session. A week later, quinidine sulfate (Hoei Yakko Co.) dissolved in the same vehicle was administered at 20 mg/kg and tested in the same manner. Between these two test sessions, the rats were trained under the mult RR 20 [food] RR 20 [shock] schedule every day except Sunday. The same two rats were used again in the kanamycin test two months later, as no residual effect of quinidine was observable.

The drug test sessions involving kanamycin were conducted with three groups of two rats each. These included a saline control group, a kanamycin low-dose group and a kanamycin high-dose group. Saline at a volume of 1 ml/kg was administered intramuscularly to the rats in the saline control group at 10:00 a.m. every day except Sundays. Kanamycin sulfate (Meiji) was administered to the rats in the kanamycin low-dose group at 250 mg/kg in the same manner as in the saline control group. The kanamycin high-dose group received an additional dose of 250 mg/kg at 4:00 p.m. The other experimental conditions were the same as in the kanamycin low-dose group. For each rat the drug test session was conducted once every 2 to 10 days at noon over several weeks. Between test sessions the rats were trained on the mult RR 20 [food] RR [shock] schedule every day except Sunday. The threshold test session was conducted again one month after the last saline or kanamycin administration. Between the last administration and the following threshold test session, rats were trained under the RR 20 schedule for food every day except Sunday.

RESULTS

After lever pressing under the continuous reinforcement schedule had been established, 8–12 sessions were required to obtain stable responding under the RR 20 schedule for food. After 76–111 sessions of training under the mult RR 20 [food] RR 20 [shock] schedule, a stable and high rate of responding (45.7–95.7 R's/min) during the food periods as well as conditioned suppression during the stimulus w/shock periods (SR>0.99) was observed in all rats. In the threshold test, the intensity of the tone initially decreased from 60 dB by several steps and then oscillated around certain values (e.g., 20–30 dB with R 504, see Fig. 1). This stabilized track-

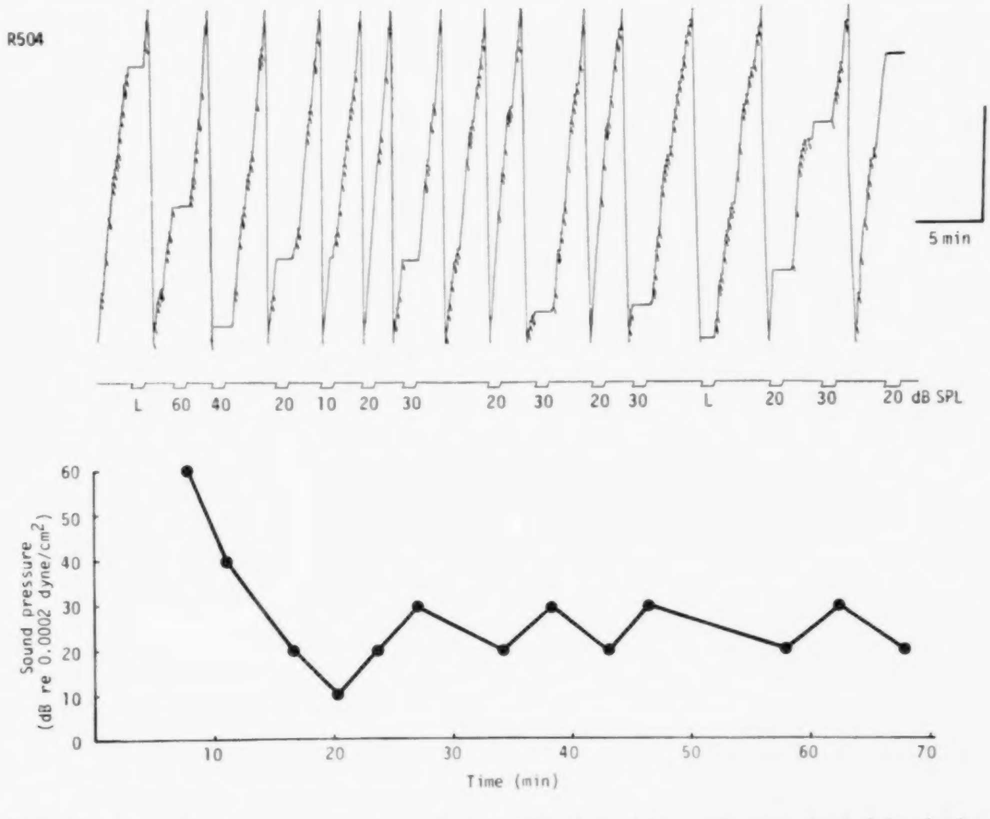

FIG. 1. Sample cumulative response record (upper panel) and tracking of the sound pressure level of the stimulus tone (lower panel) by Rat No. 504 in the threshold test. The sound pressure level, indicated under the offset of event pen in the upper panel, was successively decreased when the conditioned suppression was observed, or increased when the conditioned suppression was not observed. A light, indicated as L under the offset of event pen, was used as the control stimulus. The data points in the lower panel correspond to the sound pressure levels directly above in the upper panel. After several presentations of the tone, the levels oscillated between 20 and 30 dB.

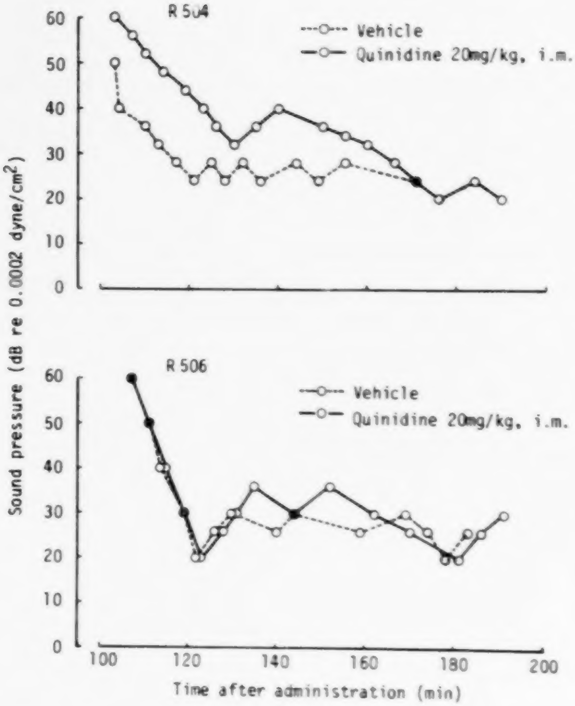

FIG. 2. Effect of intramuscular administration of quinidine at 20 mg/kg to rats on the tracking of the stimulus tone sound pressure level. Compared to the vehicle, the levels in both rats increased with quinidine.

ing within a range of less than 10 dB was observed 20–30 min after the start of the session in all rats.

In all rats, neither the level nor the range of the stabilized tracking were affected by administration of the vehicle. With quinidine at 20 mg/kg, the level of the tracking was higher than for the vehicle between 100 min and 160 min after administration in R 504 and between 135 min and 160 min after administration in R 506 (Fig. 2). In both rats, the level recovered to the vehicle level about 170 min after administration. The conditioned suppression with the control light was not attenuated either after vehicle administration or after quinidine administration in either rat (SR >0.95).

The effects of repeated administration of kanamycin on auditory threshold are presented in Fig. 3. The threshold was herein defined as the minimum sound pressure level of the tone after the 4th presentation of the tone in the session. The threshold did not change after several weeks of repeated administration of saline in either rat. However, the threshold increased during test sessions on the 15th day of administrations of 250 mg/kg/day IM of kanamycine in R 510 and on the 24th day in R 509. In the test session on the first day of administration with the kanamycin high-dose group, rats were tested at noon after the first dose of 250 mg/kg IM of kanamycin and the second dose of 250 mg/kg was not given until 4:00 p.m. R 506 failed to respond during the test session after this first administration of kanamycin. With kanamycin at 500 mg/kg/day IM, the threshold increased on the 13th day in R 506 and on the 19th day in R 508. One month after the last administration, the threshold was still at the same level as that of the last administration for each rat. Throughout the test sessions with all the rats in the kanamycin test, the conditioned suppression was generally not attenuated by the control light (SR >0.90). However, the SR for the control light in one out of two stimulus periods in the test session was sometimes less than 0.90 in all rats of both the kanamycin low- and high-dose groups.

EXPERIMENT 2

The effects on visual threshold of the application of pilocarpine to both eyes and of the subcutaneous administration of LSD-25 were tested in rhesus monkeys using a trialwise tracking method similar to that used in Experiment 1.

METHOD

Subjects

The same two male rhesus monkeys were used in both the pilocarpine test and the LSD-25 test. They had been used for the conditioned emotional response experiment after Estes and Skinner [11]. Their body weights at the start of the experiment were 6.0 kg for M239 and 7.1 kg for M543. Both monkeys were housed in individual living cages. They were fed a total of 80 g of food per day throughout the experiments.

Apparatus

During the experimental session, the monkeys were restrained in a primate chair in a darkened experimental room. In front of the monkey was a panel which contained a lever, a food tray, and a red lamp which was used as a stimulus light. The lever was made of stainless steel (5 × 5 × 0.3 cm) and required a minimum force of 50 g to operate. The food tray was placed 7 cm under the lever and was dimly illuminated by a small lamp. The red lamp (dia. 1.5 cm) was located 20 cm away from a point midway between the monkey's eyes. A pellet dispenser (Ralph Gerbrands Co., Model A) was used to dispense a soybean (about 0.2 g) into the food tray. The luminance of the stimulus light was changed by adjusting the voltage supplied to the red lamp by manual operation of the dial of a variable resistor. The luminance of the red lamp at each voltage was calibrated by a Pritchard photometer (Photo-Research Co., Spectra), the data values being represented in millilamberts (mL). An interrupted pure tone (4 KHz) as the control discriminative stimulus (the control tone) was generated by a home-made oscillator through two speakers each located 30 cm away from the monkey's ears. The tone was presented for 0.5 sec intervals separated by 0.5 sec intervals of silence. Masking noise was presented throughout the sessions through the same speakers. Neither the intensity of the tone nor of the masking noise was calibrated; however, the tone could easily be detected through the masking noise. Three mA of electric shock were delivered to the monkey's tail. The experimental contingencies and data recording were arranged as in Experiment 1.

Procedure

Training under the multiple schedule. Monkeys were trained under the same schedule as in Experiment 1 (i.e., the mult RR 20 [food] RR 20 [shock] schedule). Each training

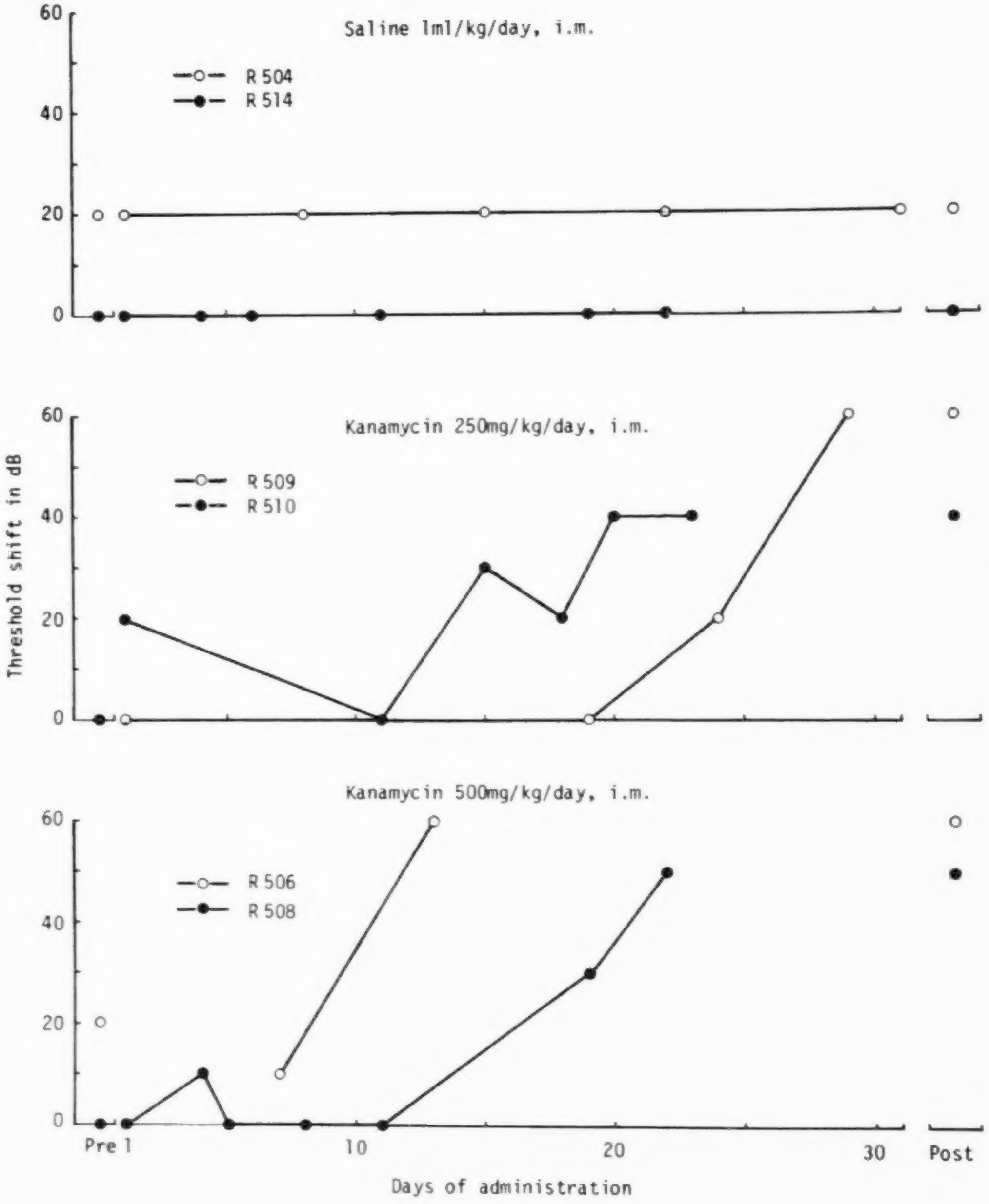

FIG. 3. Effect of intramuscular administration of kanamycin on the auditory threshold in rats. The threshold was defined as the minimum sound pressure level after the 4th presentation of the tone in the session. By repeated administration, kanamycin at 250 mg/kg/day and at 500 mg/kg/day increased the threshold level while saline did not. One month after the last administration of kanamycin, the increased threshold levels were still maintained (data points marked "Post").

session included 24 stimulus w/shock periods. In the 3rd, the 11th, and the 19th stimulus w/shock periods, the control tone was used while in the other periods, the light (a red lamp supplied with 50 VAC) was used. In this training as well as in the threshold test and the drug tests described below, the monkey was kept in the primate chair and dark-adapted to the experimental room for 20 min before the session. The other procedures for the training under the multiple schedule were the same as in Experiment 1.

Threshold test. After both a stable, high rate of responding during the food periods and suppression of responding in the stimulus w/shock periods were observed, the visual threshold test sessions were given in the same manner as in Experiment 1 except that the intensity of the test light was changed by adjusting the voltage to the lamp by either 1, 2.5, 5, or 10 V steps. The threshold for luminance was determined in the same manner as in Experiment 1. The intensity of the tone was kept constant throughout Experiment 2.

Drug tests. After consistent and reproducible tracking data were obtained in the threshold test, the effect of the drug was tested. In the pilocarpine test, saline was applied to both eyes at a volume of 0.15 ml per eye 25 min prior to the test session. Following this, pilocarpine hydrochloride (Mohan Yakuhin Co.) dissolved in saline was tested at several doses once a week in the same manner as in the saline test session. The doses tested were 0.16 mg/kg in M239, and 0.02, 0.04, 0.08, and 0.16 mg/kg in M543. The pupil size of the left eye was measured in both monkeys after each test session. Between test sessions, monkeys were trained under the mult RR 20 [food] RR 20 [shock] schedule every day except Sunday.

In LSD-25 test sessions, the vehicle, tartaric acid diluted in saline at a concentration of 0.0048% was administered subcutaneously at 0.5 ml/kg, 25 min prior to the test session. Each dose of LSD-25 dissolved in the vehicle was tested once a week in the same manner as the vehicle test session. The doses tested were 10, 20 and 30 μg/kg in M239, and 2, 4, and 8 μg/kg in M 543. In the days following the LSD-25 test sessions, the threshold test session was given again without any administration to check the effect of LSD-25 after 24 hr. Between drug test sessions, the monkeys were trained under the mult RR 20 [food] RR 20 [shock] schedule every day except Sunday. The interval between the pilocarpine test and the LSD-25 test was 2 weeks or longer.

RESULTS

After 10 sessions of training under the RR 20 schedule for food and 10–13 sessions of training under the mult RR 20 [food] RR 20 [shock] schedule, a stable and high rate of responding (118.5–158.2 R's/min) during the food periods as well as conditioned suppression during the stimulus w/shock periods (SR >0.99) were observed in both monkeys. In the threshold test, the luminance of the light initially decreased from 0.64 log mL (50 VAC) by several steps and then oscillated around certain values (e.g., −3.79 to −4.88 log mL in M239 and −4.04 to −4.82 log mL in M543). The levels and the range of the stabilized tracking were not affected by administration of the vehicle in either the pilocarpine test or in the LSD-25 test in either monkey.

Pilocarpine applied to the eyes decreased the pupil size. The pupil sizes were 5.0 mm in M239 and 4.5 mm in M543 after saline administration and decreased to 1.0 mm at 0.16 mg/kg in M239, and for M543, 1.5 mm at 0.02 mg/kg, 1.3 mm at 0.04 mg/kg and 1.0 mm at 0.8 and 1.6 mg/kg of pilocarpine. As shown in Fig. 4, the levels of the tracking for the light were slightly higher after pilocarpine administrations than after saline administration. The levels of the tracking after pilocarpine at 0.04 and at 0.16 mg/kg in M543 are not presented in Fig. 4 as they were almost identical to that of pilocarpine at 0.08 mg/kg.

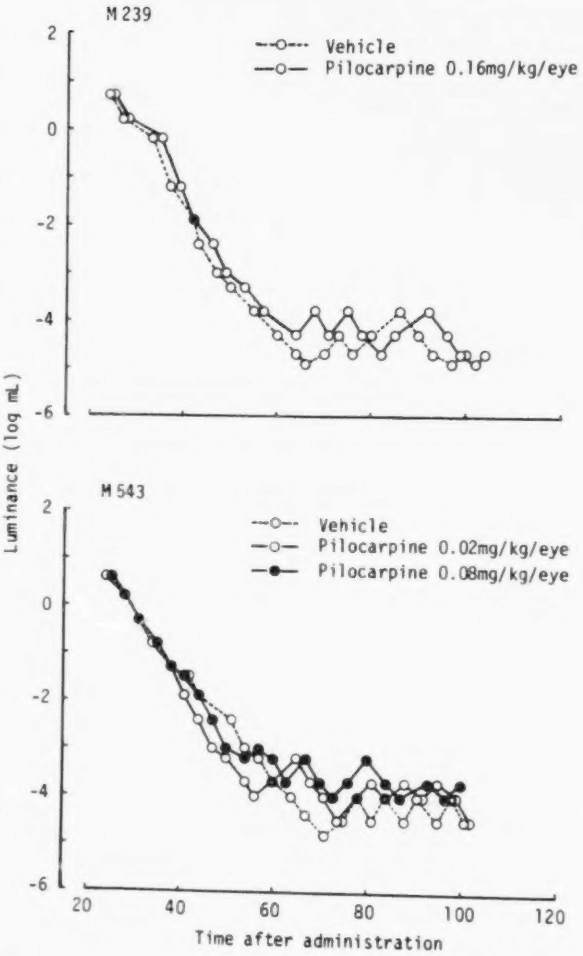

FIG. 4. Effect of application of pilocarpine to the eyes of rhesus monkeys on the tracking of the luminance of the stimulus light. Compared to the vehicle, pilocarpine slightly increased the levels in both monkeys.

Subcutaneous administration of LSD-25 increased the tracking levels at 20 and 30 μg/kg in M239 and at 4 μg/kg and 8 μg/kg in M543 (Fig. 5). The increased levels at 4 and 8 μg/kg in M543 recovered to the vehicle level 24 hr after administration. Although data are not shown, no remarkable difference in the levels of the tracking compared with the vehicle levels was observed at 10 μg/kg in M239 or at 2 μg/kg in M543.

In both the pilocarpine test and the LSD-25 test with the two monkeys, the conditioned suppression for the control

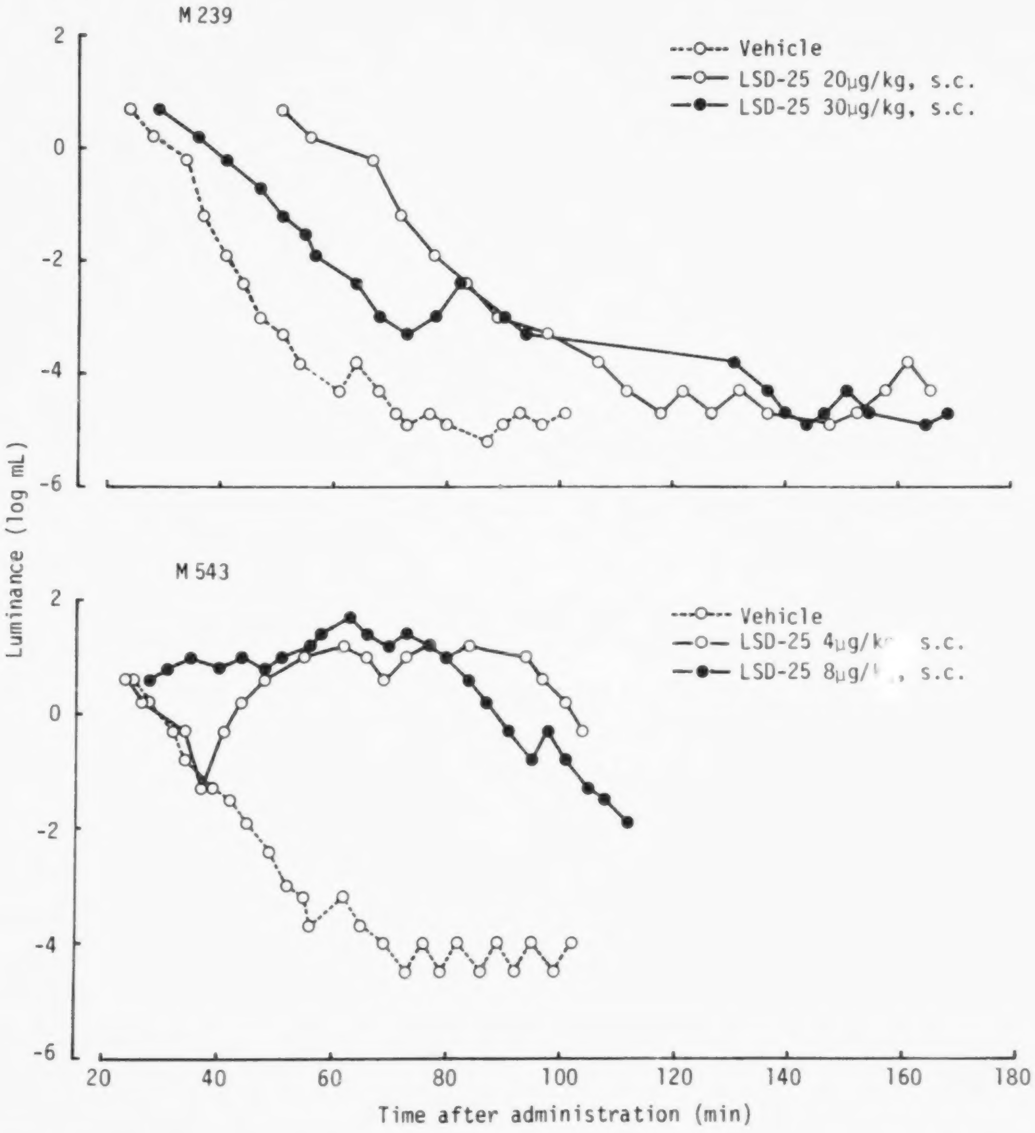

FIG. 5. Effect of subcutaneous administration of LSD-25 on ths tracking of the luminance of the stimulus light in rhesus monkeys. Compared to the vehicle, LSD-25 increased the levels in both monkeys.

tone was not attenuated either after vehicle administration or after drug administration (SR >0.95 except in LSD-25 at 20 μg/kg in M239 where SR was 0.89).

DISCUSSION

One of the advantages of the tracking method lies in its power to assure reliability with a relatively small set of data points. However, the tracking method may require long training sessions and complex apparatus. A combination of a tracking procedure and a conditioned suppression paradigm seems to produce some advantages both because conditioned suppression may be well established and because the baseline under this paradigm is stable within each session and over a series of sessions. As the presentation of the stimulus at a certain intensity is given trialwise in the present tracking procedure, the experimenter has enough time to adjust the dial of the stimulus generator manually. An apparatus providing automatic variation of stimulus intensity is not indispensable with trialwise tracking as it is with continuous tracking [5].

In the present experiments, the levels of tracking in the threshold tests were stabilized within a narrow range after several stimulus presentations. This tracking range was stable for each animal over all threshold test sessions (e.g., the ranges of the stabilized tracking were 10 dB in Experiment 1 and 1 log mL in Experiment 2). To ensure that the present method was specifically evaluating sensory threshold, the effect of eardrum lesions was tested in rats. In the threshold test just after the lesion, both a high rate of responding during the tone presentation periods and complete conditioned suppression during the light presentation periods were observed, and the tracking levels in this test session were above 50 dB. When the threshold test session was given in the darkened room to a non-dark-adapted monkey, the levels of tracking were higher than in the test sessions with dark adaptation, while stabilized tracking and complete suppression to the tone were observed. This evidence with rats and monkeys may indicate that the present method is valid enough to measure sensory threshold specifically.

In the drug tests, both single administrations of quinidine and repeated administrations of kanamycin increased auditory threshold in rats, and single administrations of pilocarpine or of LSD-25 increased visual threshold in rhesus monkeys. As the conditioned suppression to the control stimulus was not markedly attenuated in the present experiments, the action of drugs tested were different from benzodiazepines which have general attenuating effects on conditioned suppression [7,14].

The auditory threshold change with quinidine observed in rats was in agreement with the change previously observed in a monkey [27]. While the effect of quinidine was reversible, the effect with repeated administration of kanamycin was irreversible, because hearing loss with kanamycin is caused by the loss of receptor cells of the cochlea [26]. Auditory threshold changes with kanamycin were detected on the 13th day at 500 mg/kg and the 20th day at 250 mg/kg. Gourevitch [16] detected changes after several months of repeated administration of kanamycin at 100 or 200 mg/kg/day in rats, and Stebbins [26] after two months of repeated administration of kanamycin at 100 mg/kg/day to monkeys. The visual threshold changes with LSD-25 in monkeys were more marked than those produced by the miotic agent, pilocarpine. Threshold increase with LSD-25 was also reported by Blough [3] for pigeons at 100 μg/kg PO or IP. Thus it can be seen by comparison of the above with the results reported in the present experiments, that in the kanamycin test our procedure effected a saving of time, while in the LSD test the drug effect was observed at a considerably lower dose, although in the latter case the species difference of the test animals must also be considered. The trialwise tracking method used was sensitive enough to test the selective effect of drugs on sensory thresholds and was also a relatively easy way to obtain a stable behavioral baseline for experimental purposes.

REFERENCES

1. Akiyoshi, M. and K. Sato. Reevaluation of the pinna reflex test as screening for ototoxicity of antibiotics. In: *Proceedings of the 6th International Congress of Chemotherapy*. Tokyo: University of Tokyo Press, 1970, pp. 621–627.
2. Anderson, H. and E. Wedenberg. A new method for hearing tests in the guinea pig. *Acta otolar.* **60:** 375–393, 1965.
3. Blough, D. S. Effect of lysergic acid diethylamide on absolute visual threshold of the pigeon. *Science* **126:** 304–305, 1957.
4. Blough, D. S. Some effects of drugs on visual discrimination in the pigeon. *Ann. N.Y. Acad. Sci.* **66:** 733–739, 1957.
5. Blough, D. and P. Blough. Animal psychophysics. In: *Handbook of Operant Behavior*, edited by W. K. Honig and J. E. R. Staddon. Englewood Cliffs, New Jersey: Prentice-Hall, Inc., 1977, pp. 514–439.
6. Chiba, S. and K. Ando. Effects of chronic administration of kanamycin on conditioned suppression to auditory stimulus in rats. *Jap. J. Pharmac.* **26:** 419–426, 1976.
7. Cook, L. and A. B. Davidson. Effects of behaviorally active drugs in a conflict-punishment procedure in rats. In: *The Benzodiazepines*, edited by S. Garattini, E. Mussini and L. O. Randall. New York: Raven Press, 1973, pp. 327–345.
8. Courvoisier, S. and O. Leau. Study of the onset of deafness in rats treated with streptomycin, dihydrostreptomycin, and neomycin. *Antibiotics and Chemother.* **6:** 411–420, 1956.
9. Crifò, S. Shiver-audiometry in the conditioned guinea-pig (Simplified Anderson-Wedenberg test). *Acta otolar.* **75:** 38–44, 1973.
10. Darcy, P. F. and J. P. Griffin. *Iatrogenic Disease*. London: Oxford University Press, 1972, pp. 151–164.
11. Estes, W. K. and B. F. Skinner. Some quantitative properties of anxiety. *J. exp. Psychol.* **29:** 390–400, 1941.
12. Evans, H. L. Scopolamine effects on visual discrimination: Modifications related to stimulus control. *J. Pharmac. exp. Ther.* **195:** 105–113, 1975.
13. Ferster, C. B. and B. F. Skinner. *Schedules of Reinforcement*. New York: Appleton-Century-Crofts, 1957, pp. 503–579.
14. Geller, I., J. T. Kulak and J. Seifter. The effects of chlordiazepoxide and chlorpromazine on a punishment discrimination. *Psychopharmacologia* **3:** 374–385, 1962.
15. Goodman, L. S. and A. Gilman. *The Pharmacological Basis of Therapeutics*, 5th edition. New York: The Macmillan Company, 1975.
16. Gourevitch, G. Detectability of tones in quiet and in noise by rats and monkeys. In: *Animal Psychophysics: The Design and Conduct of Sensory Experiments*, edited by W. C. Stebbins. New York: Plenum Press, 1970, pp. 67–97.
17. Hanson, H. M. Psychophysical evaluation of toxic effects on sensory systems. In: *Behavioral Pharmacology: The Current Status*, edited by B. Weiss and V. G. Laties. New York: Plenum Press, 1976, pp. 195–205.
18. Hearst, E. Drug effects on stimulus generalization gradients in the monkey. *Psychopharmacologia* **6:** 57–70, 1964.
19. Heffner, R., H. Heffner and B. Masterton. Behavioral measurements of absolute and frequency-difference thresholds in guinea pig. *J. acoust. Soc. Amer.* **49:** 1888–1895, 1971.
20. Laties, V. G. and B. Weiss. Influence of drugs on behavior controlled by internal and external stimuli. *J. Pharmac. exp. Ther.* **152:** 388–396, 1966.
21. Laties, V. G. The modification of drug effects on behavior by external discriminative stimuli. *J. Pharmac. exp. Ther.* **183:** 1–13, 1972.
22. Ray, A. R. Psychophysical testing of neurologic mutant mice. In: *Animal Psychophysics: The Design and Conduct of Sensory Experiments*, edited by W. C. Stebbins. New York: Plenum Press, 1970, pp. 99–124.
23. Reiter, L. W., G. M. Talens and D. E. Woolley. Parathion administration in the monkey: Time course of inhibition and recovery of blood cholinesterases and visual discrimination performance. *Toxic. appl. Pharmac.* **33:** 1–13, 1975.
24. Rosenberger, P. B. Response-adjusting stimulus intensity. In: *Animal Psychophysics: The Design and Conduct of Sensory Experiments*, edited by W. C. Stebbins. New York: Plenum Press, 1970, pp. 161–184.
25. Rosenberger, P. B. and J. T. Ernest. Behavioral assessment of absolute visual thresholds in the albino rat. *Vision Res.* **11:** 199–207, 1971.
26. Stebbins, W. C. Studies of hearing and hearing loss in the monkey. In: *Animal Psychophysics: The Design and Conduct of Sensory Experiments*, edited by W. C. Stebbins. New York: Plenum Press, 1970, pp. 41–66.
27. Stebbins, W. C., W. W. Clark, R. D. Pearson and N. G. Weiland. Noise- and drug-induced hearing loss in monkeys. *Adv. Otorhino-laryng.* **20:** 42–63, 1973.
28. Sidman, M., B. A. Ray, R. L. Sidman and J. M. Klinger. Hearing and vision in neurological mutant mice: A method for their evaluation. *Expl Neurol.* **16:** 377–402, 1966.

Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function
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Motor Activity: A Survey of Methods with Potential Use in Toxicity Testing

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REITER, L. W. AND R. C. MACPHAIL. *Motor activity: A survey of methods with potential use in toxicity testing.* NEUROBEHAV. TOXICOL. **1**: Suppl. 1, 53–66, 1979.—Activity measurements are expected to have widespread use in toxicity testing. The multifaceted nature of motor activity will directly influence the selection of a measurement technique since the relative contribution of various motor acts to any particular measurement will depend upon the detection method. Because of the apparatus-dependent nature of motor activity measurements, it is recommended that consideration be given to how accurately the various devices measure locomotor activity. In the present paper, two types of body movement will be considered as locomotor activity: ambulation (horizontally directed movement) and rearing (vertically directed movement). Discussion focuses on the various methods currently used to record motor activity, the various components of motor activity which are likely to be recorded, and the advantages and disadvantages of these techniques for the measurement of locomotor activity. Finally, consideration is given to studies which have compared treatment effects on motor activity derived from two or more measurement techniques.

Motor activity   Toxicity testing   Measurement technique

THE Toxic Substances Control Act specifically includes behavioral disorders as a health effect for which standard test data are to be developed. Behavioral testing will be used to evaluate both new substances and substances currently in commerce. Although it is likely that the strategy for these two test situations may be quite different, the actual test systems employed may overlap. New substances need to be tested for their neurotoxic properties. In this case, a series of behavioral tests is needed which will provide a reliable forecast of neurotoxicity. The greatest concern here is that a neurotoxicant not go undetected. On the other hand, testing of substances currently in use should focus on more clearly defining the neurotoxicity and should, therefore, be concerned with characterizing the types of behavioral changes associated with exposure along with the factors which influence these changes. Nevertheless, in both situations, there is a need for behavioral test systems which are reliable, sensitive and cost-effective.

It is very likely that motor activity will be widely used in evaluating toxic substances, see [44, 79, 88]. In the present paper, discussion of locomotor activity will focus on two types of body movement: ambulation (horizontal movement) and rearing (vertical movement). Next we will consider measurement techniques that are used with rodents including both observational and automated techniques. Consideration will be given to the behavioral components that contribute to different measures of activity including the fidelity with which the devices measure locomotor activity. Finally, we will examine the issue of cross reliability by reviewing experiments which compared two or more measures of motor activity.

GENERAL MOTOR ACTIVITY VS LOCOMOTOR ACTIVITY

Motor activity is not a unitary class of behavior. The terms "general motor activity" and "spontaneous motor activity" refer to the numerous motor acts, occurring either alone or in combination, which constitute an animal's behavioral repertoire. Quantitation of the general activity level of a rodent requires measurement of the total frequency of acts, such as walking, rearing, sniffing, grooming, etc. Because of the heterogeneous nature of general activity, it is doubtful that a single measure could ever be developed. Skinner [105], recognizing this point, concluded that "no attempt is made to distinguish between the various forms that the activity may take. Since each form should have its own units, a quantitative measure of activity as a whole is practically impossible."

Many investigators have utilized observational techniques to quantitate general activity. Draper [24], for example, developed an extensive list of descriptive behavioral units which he used to record the home-cage activity of rats (see Table 1). These behavioral units were divided into three categories: ambulatory, non-ambulatory and inactivity. The extensive (albeit incomplete) nature of this list should be cause for concern when one considers that a toxicant could differentially affect the various components of an animal's general activity. Norton [70], for example, used time-lapse photography to examine the effects of amphetamine on 15 components of general activity in rats. Her results demonstrated (see Table 2) that amphetamine produced a differential effect on the frequency of occurrence of various motor

TABLE 1

BEHAVIORAL COMPONENTS OF HOME CAGE ACTIVITY OF RATS

Ambulatory Movements	
(a) Vigorous	
(1) Chase tail	—Rapid circular movement with tail in or near mouth
(2) Climb	—Movement across sides or roof of cage, all four feet off floor
(3) Jump	—Quick upward jump, all feet leave floor
(4) Roll over	—Turn onto back or completely over
(5) Run	—Rapid movement across floor of cage
(b) Non-Vigorous	
(1) Circle	—Slow circular shifting of position similar to that seen in dogs or cats prior to sleep
(2) Stand on cage	—Front feet raised off floor, placed on sides of cage
(3) Rear	—Front feet raised off floor or held close to body
(4) Stretch	—Back arched, front, rear legs extended
(5) Walk	—Any leg movement that moves animal slowly across floor, roof or sides of cage
Inactivity	
(a) "Relaxed" position	
(1) Head under	—Head tucked under front feet, forehead on floor
(2) Side	—Head on side on floor, body curled around
(b) "Alert" position	
(1) Stomach	—Animal on stomach, head erect, immobile
(2) Stand still	—Animal stands, body off floor, immobile
Non-Ambulatory Movements*	
(a) Groom	
(1) Bite coat	—Quick bite into fur
(2) Lick coat	—Smoothing fur with tongue
(3) Genitals	—Bite or lick genital area
(4) Nails	—Chewing feet or nails
(5) Wash face	—Stroking whiskers and/or face with one or both front feet
(6) Scratch	—Quick scratch of ear or coat with hind leg
(7) Chew tail	—
(b) "Scanning" movements	
(1) Sniff	—Nose wrinkles, audible sniff
(2) Turn head	—Horizontal or vertical head movement
(c) Displaced Activity	
(1) Chew cage	—Chew bars of cage
(2) Chew chain	—Chew chain holding bottle on cage
(d) Miscellaneous movements	
(1) Shake	—Quick shake of entire body
(2) Twitch	—Small movement of portion of body
(3) Sneeze	—
(4) Yawn	—

*Ambulatory and non-ambulatory movements are not mutually exclusive.
From: Draper (Behaviour **28**: 280–293, 1967)

components of activity. Whereas behaviors such as walking and rearing increased following amphetamine, others such as grooming and sitting decreased, and still others including turning, looking (stationary head orientation) and pawing showed no consistent alteration. Since psychoactive chemicals may differentially alter the frequencies of the motor items comprising general activity, any attempt to quantitate this activity, short of recording all motor components, may be "practically impossible". Regardless of the technique which is employed to measure activity, it is essential to understand which components of motor activity contribute to that measure.

TABLE 2

TOTAL OCCURRENCES OF BEHAVIOR ACTS IN 15 MIN

	8 Saline Control Rats per Group			8 Amphetamine Rats per Group (mg/kg)		
				0.25	0.5	1.0
	A	B	C	A	B	C
Acts Increased by Amphetamine						
Bobbing	211	222	206	293*	356*	248
Rearing	1554	1785	1932	1935*	1848	2906*
Turning	153	167	117	273*	344*	365*
Walking	517	417	384	745*	926*	1139*
Acts Decreased by Amphetamine						
Eating	239	116	210	46*	28*	0*
Grooming	148	139	141	70*	19*	4*
Patting	87	85	110	44*	21*	14*
Scratching	26	39	45	18	6*	7*
Sitting	893	684	786	461*	423*	138*
Smelling	2358	2368	2377	2314	1756*	1719*
Standing	4097	1473	3936	3833*	3771*	2658*
Washing Face	219	265	338	205	159*	36*
Acts Not Consistently Altered by Amphetamine						
Head Turning	872	904	824	993	972	902
Looking	1425	1265	1189	1416	1491*	747
Pawing	60	60	37	45	45	31

*Significant difference ($p<0.05$) using *t*-test to compare 8 control rats with 8 amphetamine-treated rats observed during the same 15 min

From: Norton (Physiol. Behav. **11**: 181–186, 1973)

The multifaceted nature of motor activity impacts directly on the use of automated measurement techniques, since the relative contribution of various motor acts to any particular measurement of activity will depend upon the detection method. In this regard, Draper [24] cautioned that the use of automated techniques has, on occasion, caused confusion since the concept of activity has become apparatus bound, and hence provides no common unit for purposes of comparison.

One major aspect of general motor activity is locomotor activity which, in the strictest sense, is defined as movement from one place to another. This movement can be either horizontally directed ambulation, including walking and running, or vertically directed including rearing, climbing, and jumping. In this discussion locomotor activity will include these two types of movement although in most experimental situations vertically directed movement is restricted to rearing.

METHODS USED TO MEASURE MOTOR ACTIVITY

A. OBSERVATIONAL TECHNIQUES

To some extent, the direct observation of behavior should always be included in toxicity testing. There will always be a need for information on the animal's general condition. The initial use of direct observation may provide important information on the toxicological properties of a compound which in turn increases the likelihood of detecting behavioral changes that may otherwise go unnoticed [39]. Toman and Everett [126], for example, noted the characteristic hunchback posture of reserpine-treated rats which would have gone undetected by most automated activity monitoring devices. The important question here, however, concerns the degree to which observational techniques may be employed in critically evaluating toxicant-induced changes in behavior.

Observational techniques employ either quantitative or qualitative measurements (Table 3). With the quantitative approach, the frequency, duration and/or sequencing of various motor components of behavior are measured. Norton, Mullenix and Culver [72], for example, have used this technique to evaluate activity in rats that had sustained brain damage from x-irradiation, carbon monoxide and pallidal lesions. In their study, the frequency and duration of 15 motor acts and the sequencing of pairs of acts were determined. An intriguing finding was that disruptions of motor sequencing were directly correlated with increases in photocell-activity counts, indicating that the increased activity measured in a photocell cage reflected a loss of the normal sequencing of behavior.

The qualitative approach is used to collect data on the presence or absence of certain components of activity. This approach is best exemplified by check lists and rating scales which have been developed by various investigators [45,135]. Use of these qualitative observational techniques has been limited primarily to the study of the acute effects of psychoactive drugs. They are well suited for identifying

TABLE 3

OBSERVATIONAL TECHNIQUES

A. Type of Measurement
  1. Quantitative — measures the frequency, duration, and/or sequencing of various motor components of behavior
      Norton (1973), Norton et al. (1976)
      Premack (1965)
  2. Qualitative — measures the presence or absence of certain motor components of behavior, i.e. check lists or rating scales
      Weismann et al. (1966)
      Irwin (1968)

B. Type of Environment
  1. Home cage
      Draper (1967)
  2. Novel environment, i.e. open field, Y-mazes, etc.
      Hall (1934)
      See Reviews by Walsh and Cummins (1976) and Archer (1973)

TABLE 4

CLASSIFICATION OF OPEN-FIELD-DEPENDENT PARAMETERS

I. Behavior
  A. Whole or Major Body Movement
    1. Type of movement
      a. Distance covered per unit time
      b. Time spent in ambulation
      c. Rearing frequency
      d. Escape attempts
      e. Latency (usually time taken to leave start area)
      f. Time spent without movement
    2. Locations
      a. Field area visited (inner or peripheral areas, corners, etc.)
      b. Affiliation (distance from partner subject)
      c. Stimulus interaction (e.g., distance from stimulus object)
  B. Part Body Movement
    1. Manipulation of objects
    2. Sniffing
    3. Scratching
    4. Digging
    5. Teeth chattering
    6. Grooming
    7. Vocalization
    8. Visual exploration

II. Autonomic Nervous System
  A. Defecation
  B. Digestive Transit Time
  C. Urination
  D. Heart Rate and Rhythm
  E. Respiratory Rate

III. Adrenal Activity
  A. Adrenal Ascorbic Acid
  B. Serum Corticosteroids

IV. Electrophysiology
  A. Hippocampal Theta Activity
  B. Electromyogram Activity

From: Walsh and Cummins (Psychol. Bull. 83: 482–504, 1976). Copyright (1976) by the American Psychological Association. Reprinted by permission.

gross changes in behavior such as tremors, ataxia, or stereotypies.

However, the use of observational techniques to detect subtle changes in behavior has three major limitations. (1) The techniques require considerable commitments of time and manpower for the data-collection process. Depending on the precision to which general activity is characterized, more than one observer may be required [103]. (2) Because of possible subjective influences on the data-collection process, a considerable degree of technical competency is required to insure reliability. The problem of interobserver reliability is especially important when interlaboratory comparisons are to be made. In this case, a minimal requirement is that strict, objective criteria are used to identify the various motor components of activity. (3) When direct observation of behavior is employed, subject-observer interaction may be an important consideration. The mere presence of the observer may modify the animal's behavior. For example, McCall *et al.* [64] demonstrated that the distribution of a rat's activity in an open field tended to concentrate on the side closest to the observer.

The use of video-tape recordings or time-lapse photography has increased considerably in observational analysis. In most instances, this has minimized the problem of subject-observer interaction and also has provided a permanent record of behavior which can be used for standardizing observations, both within and between laboratories. Recent advances in computer-automated pattern-recognition techniques have also been applied to behavioral analysis [73]. Since rigidly defined criteria are required for computer-automated identification of the various motor components of behavior, this has alleviated somewhat the problems of subjectivity and labor-intensive data collection discussed above.

Experiments using observational techniques to measure motor activity frequently remove the animal from the home cage and observe its behavior in a novel environment. The complexity of this environment has ranged from a flat surface to a simple cubicle (resembling the home cage) to complex mazes consisting of interconnecting alleys.

The most extensively employed test environment has been the open field (for review see [3,132]). Much of its popularity is almost certainly due to the simplicity of the apparatus. With few exceptions, an open field is defined as an arena that is considerably larger than the cage used to house the animal [91].

Table 4, taken from a review by Walsh and Cummins [132], lists the dependent variables which have been measured in the open field. The most widely recorded classes of behavior include ambulation (often distinguished on the basis of whether ambulation occurs in the inner or peripheral areas of the arena), frequency of rearing and grooming, and latency to first crossover. Typically, the floor of the open field is marked off into a grid arrangement and ambulation is measured by the number of times an animal enters a new square (crossovers).

The simplicity of design and ease of use of the open field almost certainly insure its continued use in behavioral testing. However, the numerous factors, both environmental and organismic, which influence open field measurements (e.g., size, illumination, species, sex and experience) require that investigators extensively report the methods employed. Surprisingly little consideration has been given toward standardization of the open field. Walsh and Cummins ([132], p. 493) summarized the problem as follows:

"Almost every physical characteristic of the apparatus, its surroundings and every procedural step have been widely varied, so that although standardization may have been established within individual laboratories, there is a disturbing lack of conformity in procedure and results within the literature as a whole.

In fact, it is hard to think of any facet which has not been modified. This difficulty of standardization is compounded by the extreme rarity of reports which cite details of more than a small proportion of relevant procedural variables."

Observational techniques have also been used with rodents tested in a complex environment such as a Y-maze. The maze consists of three alleys which join to form a Y. With this apparatus Steinberg and coworkers [92, 93, 111] have evaluated the effects of psychoactive drugs and past experience on ambulation (alley entrances) and rearing.

B. AUTOMATED TECHNIQUES

A variety of techniques have been employed to automatically record motor activity in rodents, the major classes of which are listed in Table 5. Photocell devices, field detectors and touch plates all provide direct measurements of activity because they measure the movement of the animal. The mechanical devices, on the other hand, provide an indirect measurement because they detect movement of the cage.

TABLE 5

AUTOMATED TECHNIQUES

- A. Photocell Measurements
  - 1. Simple environment
    - a. Horizontal (Siegel, 1946; Dews, 1953)
    - b. Vertical (Schnitzer and Ross, 1960)
  - 2. Complex environment
    - Norton et al. (1975)
    - Ljungberg and Ungerstedt (1977)
- B. Mechanical Measurements
  - 1. Stabilimeter
    - a. Jiggle cage
      - Richter (1927)
    - b. Tilt cage
      - Bousfield and Mote (1946)
      - Campbell et al. (1961)
    - c. Force platform
      - Denenberg et al. (1975)
      - Segal and Mandell (1974)
  - 2. Wheels
    - Stewart (1898)
    - Skinner (1933)
- C. Field Detectors
  - 1. Ultrasonic
    - Peacock and Williams (1962)
  - 2. Capacitance of a resonant circuit
    - Svensson and Thieme (1969)
- D. Touch plates (proximity counter)
  - Silbergeld and Goldberg (1973)

1. Photocell Devices

Photocell devices provide direct, relatively unambiguous measures of motor activity in which beams of light traverse a cage and impinge on photoreceptors. Any movement of an animal which interrupts a beam is recorded as an activity count; in most cases, the process of detection provides no feedback to the animal which may affect subsequent activity. Photocell chambers are stationary and therefore are free of momentum (carry-over) effects. The use of an infrared light source allows recording during the nocturnal period without detection by the animal. The greatest limitation of this technique is the possible contamination of the data with nonlocomotor activities (e.g., movements of the head and paws) when these occur immediately in front of a photobeam.

Photocell devices may be categorized primarily in terms of the complexity of the test environment and, secondarily, in terms of the number and orientation of the photobeams.

a. Simple test environments are typically circular or rectangular and generally measure activity with only one or a few photobeams. Siegel [101] is credited with using the first photocell cage to monitor activity. This device consisted of a rectangular box with a single photobeam oriented along the long axis. In 1953, Dews [23] employed a similar device to record the effects of pharmacological agents on the motor activity of mice (see also [108, 109, 129]). Numerous experiments have been conducted by Iversen and her colleagues on the effects of various treatments on the activity of rats in rectangular cages with parallel photobeams horizontally directed along the long axis (e.g. [18, 19, 54]). Rectangular chambers have also been employed in which one or two photobeams bisect the chamber across the short axis (e.g., [25, 60, 69]) and in which photobeams intersect at right angles (e.g., [41, 89, 127]).

Several simple circular photocell chambers have been used with a variety of photobeam arrangements (e.g., [29,133]). One chamber measures approximately 60 cm in diameter and is transected by two sets of three beams that intersect at right angles. Separate counts are available for the two banks of photobeams. The device has been used extensively to assess the effects of several chemical and nonchemical treatments on motor activity (e.g., [14, 38, 40, 110]).

although lately it has been supplanted by more sophisticated photocell chambers (see below).

There is a recurring concern over the importance of photocell placement [60,133]. Watzman *et al.* [133], for example, compared the effects of chlorpromazine on activity of mice tested with two different photobeam arrangements and tested either individually or in groups of five. When three parallel beams transected the chamber, more interruptions were recorded under control conditions from each of the two peripheral beams than from the center one. Also, the number of center beam interruptions was less than that obtained when the beam was oriented at a right angle with a second beam. Chlorpromazine decreased activity in a dose-dependent fashion, and this decrease depended on both the group size and on the particular photobeam arrangement. At low doses of chlorpromazine a significant dose-response was found for the crisscross photocell arrangement but not for the parallel arrangement. Therefore, with a given chamber configuration, the photocell arrangement may affect the sensitivity of a measurement technique.

In the pieces of apparatus described above, the orientation of the photobeams is along the horizontal plane. An alternative approach is to direct the photobeams downward to the sensors. One of the earliest devices that used this arrangement was described by Schnitzer and Ross [95] in which light from a single overhead source impinged on photocells located below a translucent plastic floor. Although the device has the advantage of using only one light source, thereby eliminating problems with photocell alignment, a possible limitation is that with too dense a concentration of photocells, beam interruptions could conceivably be produced by non-locomotor activities such as head turning, tail swishes, and defecation [55]. This limitation highlights a continuing concern with the use of photocell chambers: whereas too few photobeams would allow significant amounts of locomotor activity to go undetected, too many photobeams increase the likelihood that non-locomotor activities will contribute to the measurement.

Almost without exception, most simple photocell devices permit recording of horizontally-directed locomotor activity (ambulation) and exclude measurement of vertically-directed activity (rearing). In addition, very little information is collected regarding the spatial distribution of locomotor activity. There is at least one commercial photocell device, however, which can be used to measure both rearing and the spatial distribution of activity. The Electronic Motility Meter manufactured by Motron Products consists of a rectangular platform with a translucent plastic floor with 40 photosensors situated underneath. Infrared light is provided by a single overhead source. With an appropriate data-collection device, information can be collected on the spatial distribution of locomotor activity by recording and storing individual photobeam interruptions. In addition, the device has an adjustable array of photobeams and sensors for detecting rearing. The Motron device has been used for investigating the effects of drugs and other variables on locomotor activity (e.g. [1, 2, 4, 16, 33, 35, 36, 65, 77, 128, 130, 131]). Very few of these studies, however, have collected rearing data, and to date there has been no published investigation of treatment effects on the spatial distribution of locomotor activity. Although the device has many advantages over the other simple photocell test chambers currently in use, the same concerns regarding density of photocells that were discussed above apply here. Additionally, widespread use of the Motron chamber has been hampered by its high cost. At present, however, there has been at least one attempt to develop a similar but much less expensive prototype [80].

Adaptation to these simple test environments occurs rapidly, with animals showing initial high levels of activity that decrease to asymptotically low levels after several (e.g. 10–60) minutes. Downward trends in activity levels are also evident with repeated testing. Since asymptotic levels are characteristically low, simple test environments are well suited for detection of chemically induced increases in locomotor activity and/or retardation of the within-session habituation of activity.

b. Complex test environments. We consider a photocell chamber to be complex if it contains more than a single boundary. For example, a variety of otherwise simple photocell test chambers include an obstruction in the center area. One device is doughnut-shaped with the light source located in the center of the annulus and an array of six photosensors spaced equidistantly on the outer periphery. The device was first described by Wright *et al.* [136] and was manufactured as the Woodard Actophotometer. It has been used to investigate the effects of drugs and biochemical lesions of the central nervous system on motor activity (e.g. [14, 21, 62, 125, 137]). A related device was used by Kršiak *et al.* [57] in which light impinged on two photocells that intersected at right angles at the center of a square testing area. Access to the center of the chamber was precluded by a square translucent column, which was included to prevent excess activity counts by an animal simultaneously interrupting both photobeams at or near their point of intersection.

It is important to note that devices such as that used by Kršiak *et al.* [57] and Wright *et al.* [136] may have unique operating characteristics as a result of this central obstruction. It is frequently demonstrated, for example, that the locomotor activity of rodents tends to concentrate at the periphery of the test area (i.e., "wall-seeking" behavior or thigmotaxis which has been described by Barnett [4]; see also [59]). Since devices with central obstructions provide two boundaries for wall-seeking to find expression, it is likely that the relationship between locomotor activity and an independent variable differs from that found with devices that permit access to the center of the test area.

Support for this notion comes from an experiment by Stewart [113] on the effect of environmental complexity on locomotor activity in control and in scopolamine-treated rats. Although direct observation and not a photocell device was used to measure activity, the results illustrate how a complex boundary can modify behavior. Figure 1 shows the within-session time course of locomotor activity for control rats and rats treated with scopolamine (0.25 mg/kg). In a simple open field, scopolamine-treated rats had a higher level of activity than controls. However, when the complexity of the environment was increased by adding walls which divided the field into either two (C1) or four (C2) compartments, the scopolamine-treated animals were less active than controls. This difference in the drug response was due to both an increase in the rate of habituation of drug-treated animals and to a decrease in the rate of habituation of the controls. The rate of habituation, therefore, was a function of the complexity of the environment as well as the drug.

Norton *et al.* [71] have described a more complex photocell chamber with two continuous alleys that connect to a small central area and that form a figure eight when placed on its side. Two additional blind alleys extend from the central area and may provide stimuli such as water or a nest box. Locomotor activity in the figure-eight alleys is detected by

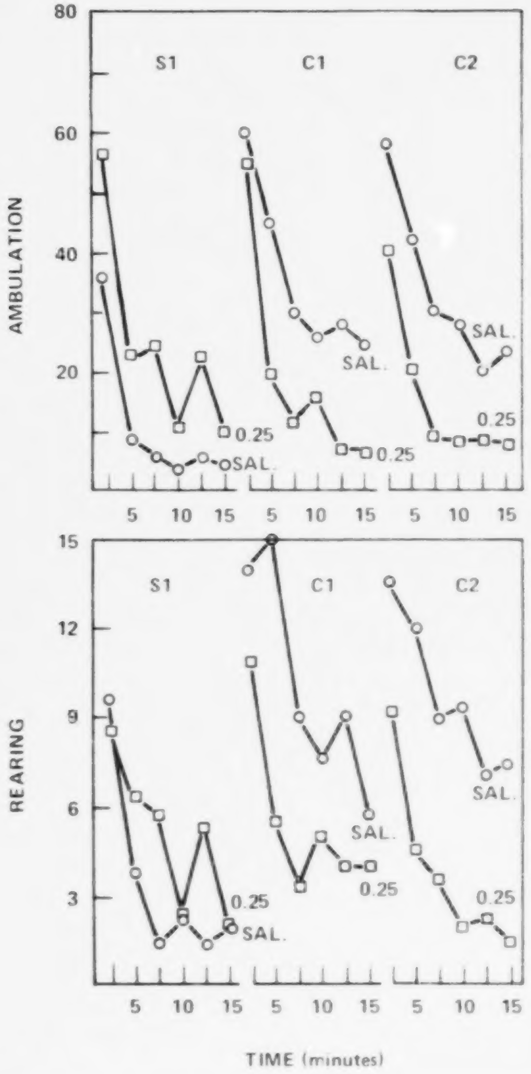

FIG. 1. Mean squares entered (ambulation) and rearing responses in saline- and scopolamine-treated (0.25 mg/kg) rats tested in three 36×36 in. environments differing in complexity (S1, simple; C1, one insert; C2, two interlocking inserts, in the field) during 2.5 min intervals of the 15 min test session. (Redrawn from: Stewart, Neurosci. Lett. **1**: 121–125, 1975.)

six pairs of photobeam and sensor and an additional beam and sensor detects excursions into each of the blind alleys. The device has been used extensively by Norton and her colleagues in studies of the effects on locomotor activity of time-of-day and social variables, as well as exposure to psychoactive drugs and environmental pollutants [71, 72, 85, 86, 87, 88].

In light of the above discussion of environmental complexity, it is interesting to compare the rate of habituation in this test chamber with habituation in the open field. Whereas Stewart [113] reported that habituation occurred within 10 min in a simple open field, this process extends over hours in the maze. These two test environments may, therefore, be expected to differ in their sensitivity to detect chemically induced alteration in the habituation process.

Ljungberg and Ungerstedt [61] have recently described a device which purports to measure several key aspects of motor activity. The outer boundary of the chamber is square with a square obstruction in the center. Horizontally oriented photobeams and sensors detect ambulation and vertically oriented beams and sensors record entries into the corners of the chamber. The floor is replete with holes below which horizontally oriented beams and sensors detect head (and other types of) entries. Finally, a microphone is used to detect gnawing behavior. Preliminary data suggest that the device is capable of recording several behavioral effects produced by drugs that alter transmission in dopamine-containing neurons. Although the device can be used to measure the spatial distribution of activity, it does not record rearing behavior. At this time, it remains to be seen whether conclusions regarding the effects of chemicals on locomotor activity obtained with this device are in any way more precise than those obtained with simpler devices.

2. *Mechnical Measurements*

The motor activity recorded with these devices involves a vertical or horizontal displacement of the chamber in response to the animal's movement. The displacement is transduced to a digital electronic signal which is counted. As with any type of mechanical measurement device, care must be taken to insure that activity counts are not confounded by momentum. Displacement of the chamber will depend in part on the age and weight of the animal. Additionally, it should be noted that mechanical devices may provide proprioceptive feedback that is positively correlated with motion.

a. Stabilimeters. Stabilimeters are so named because movements of the animal cause the chamber to be displaced from its resting position. Displacement may be in either the horizontal or vertical plane.

1. Jiggle cages. Richter [90] described a triangular activity measurement device for rats. Movements displaced the chamber vertically and this was transduced pneumatically and recorded on a revolving smoked-drum kymograph. This type of jiggle cage does not allow separate determination of rearing and ambulation, nor does it as a rule allow determination of an animal's location within the chamber. Depending on the sensitivity, non-locomotor activities may or may not be recorded.

Another type of jiggle cage, the Williamson jiggle cage, confines the animal in a very restrictive environment (approximately 15 cm in all directions). The transduction of displacements is characteristically complex [123], which makes calibration and, consequently, standardization difficult. Moreover, the device routinely detects fine-grained non-locomotor activities (e.g., tremor and face-washing) in rodents. The device has had only limited employment in the past two decades (e.g. [34, 120, 123, 124]).

Davis and Ellison [20] have described a device in which the floor of the test chamber rests on ball bearings, and movement causes the floor to displace laterally. Transduction is accomplished by closing a circuit between a metal plumb bob, that is normally centered (but not touching) within a hole in the floor just outside the chamber, and a thin film of metal that is wrapped around the rim of the hole. This device suffers from the same limitations (e.g., carry-over effects and dependence of displacement on body weight) described above for most of the mechanical measurement devices. In addition, since the device records only lateral displacements it has an undefined relationship to the more common devices which record vertical displacements.

2. *Tilt cage*. Perhaps the most widely used of the tilt cages was originally described by Bousfield and Mote [16] and subsequently modified by Campbell *et al.* [12]. A wire-mesh rectangular cage rests on a central fulcrum that runs across the chamber's width. Ambulation from one side to the other causes a slight vertical displacement of the cage which activates a microswitch. This type of tilt cage has been used extensively to characterize the effects of development, deprivation, temperature, time of day, drugs, and biochemical, electrolytic and mechanical lesions of the central nervous system (e. g. [8, 11, 27, 28, 30, 48, 49, 66, 67]). The most positive feature of this device is its simplicity and, consequently, its ease of construction and standardization. Moreover, in contrast to the jiggle cage, it is insensitive to relatively minor non-locomotor activities such as grooming and tremor. A significant limitation, however, is that some locomotor activity may go unrecorded if the animal does not cross from one side the fulcrum to the other. Also, the device does not record rearing, and provides only a crude estimate of the spatial locus of activity. Finally, although momentum (carry-over) effects are negligible, the device does provide proprioceptive stimuli as positive feedback for ambulation.

Circular tilt cages have also been described. Eayrs [26], for example, described a doughnut-shaped wire-mesh chamber that was balanced on a central pivot. Movement about the cage was detected by making and breaking electrical connections between pairs of brass contacts that were placed at 90° intervals underneath the chamber. Interestingly, in a counterbalanced crossover design, Eayrs [26] found no correlation between daily levels of activity of female rats measured in the circular tilt cage and those measured in a running wheel. Campbell [8] has also described a circular tilt cage, but it has not received widespread use.

3. *Force platforms*. Force platforms are those in which ambulatory movements cause very small displacements of the floor of the chamber. Movements are either digitized to increment counters or in other cases an analog signal is the output. Segal and Mandell [100], for example, described a chamber in which horizontal locomotor activity was recorded as crossovers on a floor that was electronically divided into quadrants. Rearing was recorded by metal touch plates located on the walls approximately 12 cm above the floor (rearing that did not include wall contact went unrecorded). That the device was relatively insensitive to small non-locomotor activities was shown in the following way. Large doses of *d*-amphetamine had a triphasic effect on activity: an initial increase in locomotor activity was followed by a period in which the rats exhibited stereotyped sequences of sniffing, grooming and gnawing. During this phase of amphetamine intoxication, ambulation and rearing were virtually absent and no activity counts were recorded. After approximately 1.5–2 hr, stereotyped behavior subsided and was accompanied by a return of locomotor activity. Although the device has many positive features, it has not been extensively used [97–100].

A particularly elegant force platform has recently been described by Denenberg and co-workers [22]. A large square test arena is supported on four corner-mounted electromechanical force transducers that provide analog outputs which are proportional to their respective supportive forces. With this device, and an appropriate data-acquisition system, it is possible to record all horizontally directed movements of the animal, however small. It is also possible to measure the spatial distribution of ambulation and, conceivably, the exact path the animal takes in moving about the chamber. Measurements have been shown to correlate well with ambulation measured by an observer [37]; it has an advantage over the open field of recording ambulation that does not result in a grid crossover [22]. It does not, however, permit registration of rearing. To date, there are no published data on the effects of chemical or non-chemical treatments on ambulation with this device and, consequently, its utility for toxicity testing remains to be determined.

b. Wheels. Running wheels are ordinarily designed so that the wheel is mounted on a horizontal axle and the animal's ambulation within the wheel causes it to rotate on its axle. The direction of rotation of the wheel may be unidirectional or bidirectional, and transduction may be accomplished in any number of ways [94, 106, 107]. Prevention of rotation with a braking device is not uncommon [78,94]. Running wheels have been used in animal behavior for over three-quarters of a century and were originally described by Stewart [112] in 1898. Skinner [106] provides an excellent description of the construction of a running wheel and the variables that influence the measurement of ambulation.

It should be obvious that ambulation recorded in running wheels involves considerable neuromuscular coordination. Also, there is considerable stimulation in the form of proprioceptive feedback, that correlates positively with the level of ambulation. The specialized nature of this activity is perhaps best reflected in the time-course for establishing stable levels of activity. In rats, activity in the running wheel typically increases with repeated testing [15, 26, 31, 91] even though the within-session trend is downward. Once asymptotic levels are reached, however, they may be very stable for long periods [15,26], although fluctuations about the mean are apparent, for example, with estrus or time of day. Large individual differences in baseline levels may be apparent [47]. Unless special precautions are taken, momentum may influence activity counts.

Running wheels have been used to study the effects of food deprivation, water deprivation, estrus, ambient temperature and time of day, lesions of the central nervous system, and a wide array of drugs on locomotor activity (e.g. [8, 32, 45–47, 51, 52, 78, 94, 96, 118, 122, 123, 134, 138]).

3. Field Detectors

Many devices have been introduced recently that record the disturbances that an organism creates in moving about a prearranged field within the test cage. Both ultrasonic and capacitive fields have been employed and each has the advantage of being stationary and, therefore, providing no feedback stimulation for activity. Measurements of activity are also unconfounded by momentum effects. The principle limitations of these techniques, however, have to do with both the degree to which locomotor activity counts may be inflated with non-locomotor activity data and the inability to adequately calibrate these devices.

a. Ultrasonic field detectors. These devices deliver an acoustic signal to the test chamber that creates a three-dimensional pattern of standing waves (e.g. [74,75]). Movements of an organism produce changes in the pattern of energy which are ordinarily transmitted to a microphone, digitized and counted. The degree of pattern interference is correlated with the speed and extent of body movement. Typically, the sensitivity of the devices can be varied over a wide range, as can the size of the test chamber. Problems arise, however, over uncertainties regarding the uniformity of the propagated field. Operating characteristics may change with fluctuations in temperature and humidity, or the deposition of fecal material. Standardization of one or more instruments is extremely tedious. Artifacts may arise through electrical interference with the signal that is sent to the counter; for this reason, coaxial cable is required. Despite the limitations described above, these types of field detectors may be of use especially when a measure of general motor activity is desired.

b. Capacitance-sensing devices. These devices utilize a tuned oscillator circuit. In the simplest case, an adjustable oscillator supplies high-frequency current to an input coil which creates a field around the test chamber. Movement within the chamber produces momentary changes in the voltage that is induced in the output coil. This signal is then amplified and an output pulse is counted. Devices of this type (e.g., Animex and Varimex) have been used extensively to evaluate drug effects (e.g. [5, 50, 52, 63, 68, 81, 104, 116, 119]). Svensson and Thieme [121] have described the uses and limitations of the Animex for recording motor activity in control and drug-treated mice. Although commercially available models are being continually refined, many of the same uses and limitations that characterize ultrasonic field sensing devices are also applicable.

4. Touch Plates

The devices in this category are included because they do not fit handily into any of the above categories. Although these devices all measure motor activity by recording contacts of the animal with sections of the chamber floor, they differ in the means used to detect these contacts.

a. Proximity counter. The floor of this device is comprised of a rectangular checkerboard arrangement of copper-clad phenolic plates that are separated by insulating material. A low-voltage oscillating current is passed between alternate plates and is adjusted so that ambulation from a ground plate to an active plate produces changes in the frequency of the oscillating current. The magnitude of this change in current is a function of the animal's capacitance, and it is this change that is typically amplified, transduced and counted. Although the principle of proximity counters is not new [139], there are very few investigations which have used them. The most notable instances are the experiments carried out by Silbergeld and her colleagues on the effect of postnatal lead exposure on the motor activity of mice (e.g. [102]). Silbergeld's device records primarily large-scale ambulatory activity; rearing would not be recorded unless it occurred concomitantly with a crossover from one to another floor plate. Although the results of these experiments have stimulated much research, no apparent attempt has been made to critically evaluate the relationship between the activity that is measured in a proximity counter and that measured in one of the more conventional devices.

b. Touch plates. These devices represent modifications of the chamber originally described by Kissel [56] and by Eayrs [26]. The basic chamber consists of a small circular alley that creates a somewhat restrictive and complex test environment for monitoring motor activity. Because of its restrictive interior, it is likely that the age and size of the rodent will determine whether ambulation is largely unidirectional or bidirectional. The original chambers were suspended on a central pivot, and in some instances delicately balanced with counter weights [26]. Movement about the chamber produced displacements that operated microswitches which in turn were used to increment counters. Subsequent modifications replaced the pivot arrangement with small floor plates that were spaced equidistantly about the cage and which rested on microswitches [83]. Although this type of chamber detects ambulation and is relatively unaffected by the occurrence of non-locomotor activities, it has no means for recording rearing. More importantly, however, its complex configuration, together with its restrictive interior, raise the possibility that the effect of a treatment may be much more complex than that determined in a larger chamber which has no central obstruction. A salient example of the complex nature of the locomotor activity recorded by this device is the dose-response curve for *d*-amphetamine [116]. In rats, increasing doses of *d*-amphetamine produce progressively greater increases in locomotor activity until a dosage of 2–4 mg/kg is reached. Further dosage increases up to about 12 mg/kg produce either no greater increase or a decrease in locomotor activity. In most instances, however, dosages of 12 mg/kg and beyond produce further increases in activity. At these heroic dosages, it is likely that a measure of ambulation reflects more the occurrence of the repetitive behavioral sequences that are produced by *d*-amphetamine than an effect of the drug on ambulation *per se*. The device has been used in several studies on the effects of psychoactive and biogenic amine-depleting drugs (e.g. [82, 83, 114–116]).

CROSS-COMPARISONS OF MOTOR-ACTIVITY-MEASUREMENT DEVICES

A limited number of studies has compared two or more measures of motor activity either in nontreated animals or in animals subjected to different types of treatments.

Tapp *et al.* [123] compared several different measures of motor activity in rats. Their results showed a lack of correlation between the various activity measures shown in Table 6. When, however, two measures were taken within the same apparatus (i.e., measures of activity within the circular field), a significant correlation was found. Otherwise, there was generally no correlation between relative levels of activity in the different test environments.

Tapp [124] has also investigated the effect of food deprivation on several measures of activity. Food deprivation did not affect activity in a Williamson jiggle cage, and decreased activity in both a simple photocell cage and a complex doughnut-shaped mechanical device (circular field). There are additional data that support the conclusion that the effects of food deprivation are apparatus-dependent. Strong [117], for example, studied the effect of food deprivation on stabilimeter activity under two conditions of measurement sensitivity. Food deprivation did not affect activity when measured in a microswitch-operated stabilimeter and decreased it when measured in a more sensitive stabilimeter device. On the other hand, Weasner *et al.* [134] obtained increases in photocell-cage activity and activity in a running wheel with food deprivation. Campbell [8] also obtained increases in activity in a running wheel and in a stabilimeter during food deprivation. The latter two studies both obtained greater increases in activity in the wheels than with the other types of measurement device. Finger [32] has also shown

TABLE 6

MATRIX OF INTERCORRELATIONS BETWEEN ACTIVITY MEASURES

Test Number	Test	1	2	3	4	5
1	Williamson Cages		−0.03	0.11	0.18	0.19
2	Photocell Cages			−0.02	−0.01	0.11
3	Activity Wheel				0.15	0.19
4	Circular Field (alternations)					0.90*
5	Circular Field (total counts)					

*$p<0.01$ Adapted from Tapp, *et al.* (Psychol. Rep. **23:** 1047–1050, 1968)

that substantially greater changes in activity occur during the estrus cycle of rats when measured in wheels than in a simple photocell device.

Animals treated with various drugs or toxicants have also been shown to respond differently when tested in different activity devices. Reiter *et al.* [86] reported changes in activity of male rats exposed to the pesticide Kepone which were apparatus-dependent. Following three weeks of treatment, rats showed a dose-related increase in locomotor activity in a figure-eight maze but a dose-related decrease in activity in an open field (Fig. 2).

The data of Kršiak *et al.* [57] illustrate further the limitations of a single measure of motor activity (Fig. 3). They examined motor activity in rats following administration of either *d*-amphetamine (dexamphetamine) or amobarbital (amylobarbitone). Animals were placed in a complex test environment (described earlier) and activity was determined simultaneously by recording photocell interruptions and by direct observation of walking, rearing and grooming. In saline-treated animals, the combined frequency of walking and rearing closely agreed with activity levels determined by photocell interruptions. In these animals, photocell counts were significantly correlated with both walking and rearing ($r=0.68$ and 0.79, respectively). However, with increasing doses of *d*-amphetamine, photocell activity counts exceeded the observationed activity. Following 1.0 mg/kg of *d*-amphetamine, walking ($r=0.82$) but not rearing ($r=0.11$) was correlated with photocell counts. On the other hand, following administration of amobarbital, there was a close agreement between photocell interruptions and observational scoring of walking and rearing.

There also appear to be discrepancies between the effects of drugs on activity measured in photocell cages and those measured in capacitance-sensing devices. Maj *et al.* [63] found that the activity of rats was about equally reduced following reserpine or α-methyl-*para*-tyrosine in both the Animex chamber and a simple photocell chamber. A large dose of *l*-dopa substantially blocked the decrease produced by reserpine and by α-methyl-*para*-tyrosine in activity measured in the Animex but not that measured in the photocell cage. Amantadine produced a dose-related blockade of the effect of reserpine on activity in the Animex chamber whereas the largest dose produced only a slight blockade of the effect in the photocell chamber. Similarly, Ljungberg [60] found that whereas a small dose of apomorphine decreased activity in both a simple photocell cage and an Animex chamber, a large dose had no effect on activity in the former device but increased it in the latter device. The effects of several other drug treatments showed no correlation between activity measurements in the two types of devices.

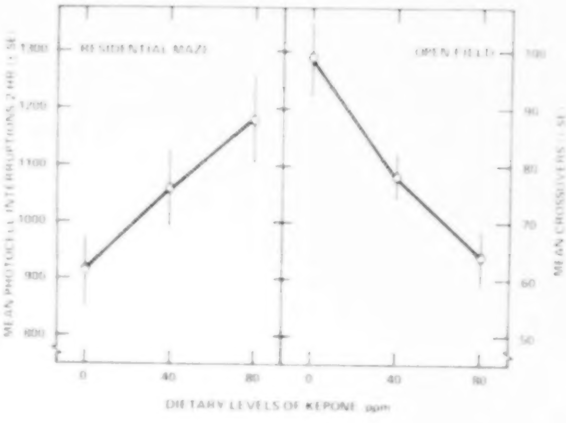

FIG. 2. Effects of dietary kepone exposure (3 weeks) on two measures of locomotor activity in the rat. Kepone exposure produced a dose-related increase in locomotor activity in a figure-eight maze but a decreased activity in an open field. (From: Reiter *et al.*, Toxic. appl. Pharmac. **41:** 143, 1977.)

Experimentally induced brain damage is another manipulation which is reported to produce apparatus-dependent changes in activity. Capobianco and Hamilton [13], for example, employed several activity measures with rats sustaining lesions of the fornix, diagonal band, and the medial forebrain bundle. Lesions of the medial forebrain bundle and diagonal band reliably increased activity in a revolving wheel whereas lesions of the fornix had a much smaller effect. Lesions of the medial forebrain bundle and the diagonal band, on the other hand, either had no effect or decreased activity in a stabilimeter whereas fornix lesions substantially increased activity. Measures of both open-field ambulation and rearing were unaffected by these lesions. This experiment suffers, however, from one possible source of experimental error which was recognized by the authors: stabilimeter testing always preceded open-field testing. It is possible that an "order effect" of testing could account for the lack of a treatment effect on activity in the open field. If, for example, lesions of the diagonal band altered the animal's reactiv-

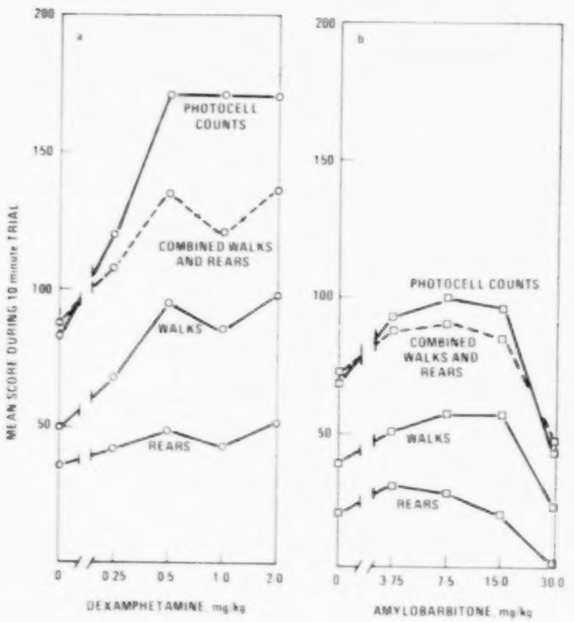

FIG. 3. Ten groups of 8 rats were injected subcutaneously either with saline or a dose of a drug 35 min before a 10 min trial in the activity cage. After dexamphetamine, marked discrepancies occurred when the results with photocells were compared with observation (Fig. 3a), but agreement was close after amylobarbitone (Fig. 3b). (Adapted from: Krsiak *et al.*, Psychopharmacology **17**: 258–274, 1970.)

ity to a novel environment, then a "carry over" effect from stabilimeter to open field would preclude detection of an effect in the latter.

Table 7 represents data obtained in adult male rats tested on successive days in a figure-eight (Residential) maze and in an open field. The order of testing was counterbalanced so that half the animals were tested first in each apparatus. Order of testing had a significant effect on activity in the open field when animals had prior exposure to the maze but the converse was not true. This potential for a carry-over effect in open-field testing has obvious implications for experiments using multiple test systems.

TABLE 7

EFFECT OF ORDER OF TESTING ON RESIDENTIAL MAZE ACTIVITY AND OPEN FIELD ACTIVITY IN ADULT RATS

	Open Field First		Residential Maze First	
Open Field (5-minutes) $t = 4.378$ 43df $p < 0.005$	67.5 ± 4.8	1st	40.3 ± 3.8	2nd
Residential Maze (5-minutes) $t = 1.33$ 43df NS	76.3 ± 8	2nd	61.3 ± 8	1st
Residential Maze (120-minutes) $t = 1.116$ 43df NS	1099 ± 67	2nd	950 ± 82	1st

CONCLUSIONS

A. Direct observations are indispensible in preliminary toxicity testing since many overt neurotoxic symptoms (i.e., tremor or ataxia) are readily detected by direct observation. The problem with this approach is that the data are not amenable to quantitative analysis.

B. Quantitative characterization of a chemical effect on motor activity can be carried out using any number of nonautomated and automated techniques. Quantitative observational techniques may be used to detect subtle changes, but they require personnel commitments, not found with automated techniques, which ultimately limit the number of animals which can be tested.

C. Many automated techniques are available for recording motor activity. The particular components of motor activity which are measured depend on the particular technique chosen. Numerous chemical and nonchemical treatments have been shown to selectively affect various components of motor activity. It is imperative, therefore, to know which components of activity a particular device is likely to detect.

D. Relationships between devices will be understood only when they are compared by measuring one or a few components of motor activity. We recommend that different devices be compared on the basis of their ability to measure locomotor activity since this represents a major component of motor activity and since most devices are designed to detect this movement. This restricted focus on locomotor activity is likely to uncover certain fundamental relationships and/or differences between the devices. We recognize, however, one possible shortcoming of this approach: since treatments may selectively affect various components of motor activity, too great a restriction on the variables to be measured may decrease sensitivity of the measure.

E. A basic requirement of activity measurements is that test methods be standardized at least within a given laboratory and that experimental details be extensively reported.

F. It is premature to conclude which of the many available activity measuring techniques is *the* most appropriate for behavioral toxicity testing. Appreciation of the relative sensitivity of the different devices will come when extensive data have been collected under standardized laboratory conditions. Future studies should, therefore, compare different measures of motor activity following exposure to neurotoxic substances.

REFERENCES

1. Ahlenius, S., N.-E. Anden and J. Engel. Restoration of locomotor activity in mice by low *l*-dopa doses after suppression by α-methyl-tyrosine but not by reserpine. *Brain Res.* **62:** 189–199, 1973.
2. Anden, N.-E., U. Strombom and T. H. Svensson. Dopamine and noradrenaline receptor stimulation: Reversal of reserpine-induced suppression of motor activity. *Psychopharmacology* **29:** 289–298, 1973.
3. Archer, J. Tests for emotionality in rats and mice: A review. *Anim. Behav.* **21:** 205–235, 1973.
4. Barnett, S. H. *The Rat: A Study in Behavior.* Chicago: Aldine Publishing Co., 1963, pp. 31–32.
5. Benkert, O. Measurement of hyperactivity in rats in a dose-response-curve after intrahypothalamic norepinephrine injection. *Life Sci.* **8:** 943–948, 1969.
6. Bousfield, W. A. and F. A. Mote. The construction of a tilting activity cage. *J. exp. Psychol.* **32:** 450–451, 1943.
7. Brase, D. A., H. H. Loh and E. L. Way. Comparison of the effects of morphine on locomotor activity, analgesia and primary and protracted physical dependence in six mouse strains. *J. Pharmac. exp. Ther.* **201:** 368–374, 1977.
8. Campbell, B. A. Theory and research on the effects of water deprivation on random activity in the rat. In: *Thirst, Proceedings from the 1st International Symposium on Thirst in the Regulation of Body Water.* Oxford: Pergamon Press, 1964, pp. 317–334.
9. Campbell, B. A. and G. A. Cicala. Studies of water deprivation in rats as a function of age. *J. comp. physiol. Psychol.* **55:** 763–768, 1962.
10. Campbell, B. A. and P. D. Mabry. The role of catecholamines in behavioral arousal during ontogenesis. *Psychopharmacology* **31:** 253–264, 1973.
11. Campbell, B. A., L. D. Lytle and H. C. Fibiger. Ontogeny of adrenergic arousal and cholinergic inhibitory mechanisms in the rat. *Science* **166:** 637–638, 1969.
12. Campbell, B. A., R. Teghtsoonian and R. A. Williams. Activity, weight loss and survival time of food deprived rats as a function of age. *J. comp. physiol. Psychol.* **54:** 216–219, 1961.
13. Capobianco, S. and L. W. Hamilton. Effects of interruption of limbic system pathways on different measures of activity. *Physiol. Behav.* **17:** 65–72, 1976.
14. Carey, R. J. and A. P. Salim. Changes in *d*-amphetamine reactivity resulting from septal forebrain injury. *Physiol. Behav.* **5:** 133–136, 1970.
15. Cornish, E. R. and N. Mrosovsky. Activity during food deprivation and satiation of six species of rodent. *Anim. Behav.* **13:** 242–248, 1965.
16. Costa, E., A. Groppetti and M. K. Naimzada. Effects of (+)-amphetamine on the turnover rate of brain catecholamines and motor activity. *Br. J. Pharmac.* **44:** 742–751, 1972.
17. Costa, E., K. M. Naimzada and A. Revuelta. Phenmetrazine, aminorex and (+)-*p*-chloroamphetamine, their effects on motor activity and turnover rate of brain catecholamines. *Br. J. Pharmac.* **43:** 570–579, 1972.
18. Creese, I. and S. D. Iversen. Blockage of amphetamine-induced motor stimulation and stereotypy in the adult rat following neonatal treatment with 6-hydroxydopamine. *Brain Res.* **55:** 369–382, 1973.
19. Creese, I. and S. D. Iversen. The pharmacological and anatomical substrates of the amphetamine response in the rat. *Brain Res.* **83:** 419–436, 1975.
20. Davis, J. D. and G. D. Ellison. A general purpose activity recorder with variable sensitivity. *J. exp. Analysis Behav.* **7:** 117–118, 1964.
21. Davis, W. M., M. Babbini, S. F. Pong, W. T. King and C. L. White. Motility of mice after amphetamine: Effects of strain, aggregation and illumination. *Pharmac. Biochem. Behav.* **2:** 803–809, 1974.
22. Denenberg, V. H., J. Gartner and M. Myers. Absolute measurement of open field activity in mice. *Physiol. Behav.* **15:** 505–509, 1975.
23. Dews, P. B. The measurement of the influence of drugs on voluntary activity in mice. *Br. J. Pharmac. Chemother.* **8:** 46–48, 1953.
24. Draper, W. A. A behavioral study of the home cage activity of the white rat. *Behaviour* **28:** 280–293, 1967.
25. Dyer, R. S., D. A. Weldon and A. H. Thoumaian. Task-specific effects of *d*-amphetamine and blindness of BALB cj mice. *Physiol. Psychol.* **4:** 175–179, 1976.
26. Eayrs, J. T. Spontaneous activity in the rat. *Br. J. Anim. Behav.* **2:** 25–30, 1954.
27. Erinoff, L., R. C. MacPhail, A. Heller and L. S. Seiden. Age-dependent effects of 6-hydroxydopamine on locomotor activity in the rat. *Brain Res.* **64:** 195–205, 1979.
28. Fibiger, H. C. and B. A. Campbell. The effect of *para*-chlorophenylalanine on spontaneous locomotor activity in the rat. *Neuropharmacology* **10:** 25–32, 1971.
29. Fibiger, H. C., C. Trimbach and B. A. Campbell. Enhanced stimulant properties of (+)-amphetamine after chronic reserpine treatment in the rat: Mediation by hypophagia and weight loss. *Neuropharmacology* **11:** 57–67, 1972.
30. Fibiger, H. C., L. D. Lytle and B. A. Campbell. Cholinergic modulation of adrenergic arousal in the developing rat. *J. comp. physiol. Psychol.* **72:** 384–389, 1970.
31. Finger, F. W. Measuring behavioral activity. In: *Methods in Psychobiology,* Vol. 2, edited by R. D. Myers. New York: Academic Press, 1972, pp. 1–19.
32. Finger, F. W. Estrus activity as a function of measuring device. *J. comp. physiol. Psychol.* **55:** 100–102, 1961.
33. Foldes, A. and E. Costa. Relationship of brain monoamine and locomotor activity in rats. *Biochem. Pharmac.* **24:** 1617–1621, 1975.
34. Freeman, J. J. and F. Sulser. Iprindole-amphetamine interactions in the rat: The role of aromatic hydroxylation of amphetamine in its mode of action. *J. Pharmac. exp. Ther.* **183:** 307–315, 1972.
35. Fuentes, J. A. and N. H. Neff. Selective monoamine oxidase inhibitor drugs as aids in evaluating the role of Type A and B enzymes. *Neuropharmacology* **14:** 819–825, 1975.
36. Fuentes, J. A., M. A. Oleshansky and N. H. Neff. Comparison of the apparent antidepressant activity of (−)- and (+)-tranylcypromine in an animal model. *Biochem. Pharmac.* **25:** 801–804, 1976.
37. Gartner, J., M. Myers, V. H. Denenberg and S. Bhat. A center-of-pressure force platform for open field activity tests. *Trans. Second New Engl. Bioengineer. Conf.*, 449–457, 1975.
38. Glick, S. D. and S. Millory. Rate-dependent effects of *d*-amphetamine on locomotor activity in mice: Possible relationship to paradoxical amphetamine sedation in minimal brain dysfunction. *Eur. J. Pharmac.* **24:** 266–268, 1973.
39. Goldstein, A., L. Aronow and S. M. Kalman. *Principles of Drug Action: The Basis of Pharmacology,* 2nd edition. New York: John Wiley and Sons, 1974, p. 735.
40. Gordon, J. H. and M. K. Schellenberger. Regional catecholamine content in the rat brain: Sex differences and correlation with motor activity. *Neuropharmacology* **13:** 129–137, 1974.
41. Grant, M. Cholinergic influences on habituation of exploratory activity in mice. *J. comp. physiol. Psychol.* **86:** 853–857, 1974.
42. Hollister, A. S., G. R. Breese and B. R. Cooper. Comparison of tyrosine hydroxylase and dopamine-β-hydroxylase inhibition with the effects of various 6-hydroxydopamine treatments on *d*-amphetamine-induced motor activity. *Psychopharmacology* **36:** 1–16, 1974.
43. Hollister, A. S., G. R. Breese, C. M. Kuhn, B. R. Cooper and S. M. Schanberg. An inhibitory role for brain serotonin-containing systems in the locomotor effects of *d*-amphetamine. *J. Pharmac. exp. Ther.* **198:** 12–22, 1976.

44. *Human Health and the Environment–Some Research Needs.* U.S. Department of Health, Education and Welfare, Public Health Service/National Institutes of Health. Washington, D.C.: U.S. Government Printing Office, 1977.
45. Irwin, S. Comprehensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. *Psychopharmacology* **13:** 222–257, 1968.
46. Irwin, S. The action of drugs on psychomotor activity. *Rev. Can. Biol.* **20:** 239–250, 1961.
47. Irwin, S., M. Slabok and G. Thomas. Individual differences: I. Correlation between control locomotor activity and sensitivity to stimulant and depressant drugs. *J. Pharmac. exp. Ther.* **123:** 206–211, 1958.
48. Jacobs, B. L., W. D. Wise and K. M. Taylor. Differential behavioral and neurochemical effects following lesions of the dorsal or median raphe nuclei in rats. *Brain Res.* **79:** 353–361, 1974.
49. Jacobs, B. L., C. Trimbach, E. E. Eubanks and M. Trulson. Hippocampal mediation of raphe lesion and PCPA-induced hyperactivity in the rat. *Brain Res.* **94:** 253–261, 1975.
50. Jenner, P. and C. D. Marsden. The influence of piribedil (ET495) on components of locomotor activity. *Eur. J. Pharmac.* **33:** 211–215, 1975.
51. Karczmar, A. G., C. L. Scudder and D. L. Richardson. Interdisciplinary approach to the study of behavior in related mice types. In: *Neurosciences Research, Vol. 5, Chemical Approaches to Brain Function,* edited by I. Kopin and S. Ehrenpries. New York: Academic Press, 1973, pp. 159–244.
52. Kavanau, J. L. and D. H. Brant. Wheel-running preferences of *Peromyscus. Nature* **208:** 597–598, 1965.
53. Kellogg, C. and P. Lundborg. Ontogenic variations in responses to *l*-dopa and monoamine receptor-stimulating agents. *Psychopharmacology* **23:** 187–200, 1972.
54. Kelly, P. H., P. W. Seviour and S. D. Iversen. Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. *Brain Res.* **94:** 507–522, 1975.
55. Kinnard, W. J. and N. Watzman. Techniques utilized in the evaluation of psychotropic drugs on animal activity. *J. Pharmac. Sci.* **55:** 995–1012, 1966.
56. Kissel, J. W. Nutating annular cage for measuring motor activity. *Science* **134:** 1224–1225, 1963.
57. Krsiak, M., H. Steinberg and I. P. Stolerman. Uses and limitations of photocell activity cages for assessing effects of drugs. *Psychopharmacology* **17:** 258–274, 1970.
58. Kuschinsky, K. Are cholinergic mechanisms involved in morphine effects on motility? *Naunyn-Schmiedeberg's Arch. Pharmac.* **281:** 167–173, 1974.
59. Lat, J. The spontaneous exploratory reaction as a tool for psychopharmacological studies. A contribution towards a theory of contradictory results in psychopharmacology. In: *Pharmacology of Conditioning, Learning and Retention,* edited by M. Mikhel'son, V. G. Longo and Z. Votava. New York: Pergamon Press, 1965, pp. 47–66.
60. Ljungberg, T. Reliability of two activity boxes commonly used to assess drug-induced behavioral changes. *Pharmac. Biochem. Behav.* **8:** 191–195, 1978.
61. Ljungberg, T. and U. Ungerstedt. A method for simultaneous recording of eight behavioral parameters related to monoamine neurotransmission. *Pharmac. Biochem. Behav.* **8:** 483–489, 1978.
62. Maickel, R. P., R. M. Levine and J. E. Zabik. Differential effects of *d*- and *l*-amphetamine on spontaneous motor activity in mice. *Res. communs. chem. pathol. Pharmac.* **8:** 711–714, 1974.
63. Mai, J., B. Durek and W. Palider. Differences in locomotor activity of mice as measured by an Animex and photoresistor actometer. *J. Pharmac. Pharmac.* **23:** 979–981, 1971.
64. McCall, R. B., M. L. Lester and C. M. Corter. Caretaker effects in rats. *Devl. Psychol.* **1:** 771, 1969.
65. Modigh, K. Central and peripheral effects of 5-hydroxytryptophan on motor activity in mice. *Psychopharmacology* **23:** 48–54, 1972.
66. Moorcroft, W. H. Ontogeny of forebrain inhibition of behavioral arousal in the rat. *Brain Res.* **35:** 513–522, 1971.
67. Moorcroft, W. H., L. D. Lytle and B. A. Campbell. Ontogeny of starvation-induced behavioral arousal in the rat. *J. comp. physiol. Psychol.* **75:** 59–67, 1971.
68. Mukherjee, B. P., P. T. Bailey and S. N. Pradhan. Correlation of lithium effects on motor activity with its brain concentrations in rats. *Neuropharmacology* **16:** 241–244, 1977.
69. Nagy, Z. M. and M. Ritter. Ontogeny of behavioral arousal in the mouse: Effects of prior testing upon age of peak activity. *Bull. Psychon. Soc.* **7:** 285–288, 1976.
70. Norton, S. Amphetamine as a model for hyperactivity in the rat. *Physiol. Behav.* **11:** 181–186, 1973.
71. Norton, S., B. Culver and P. Mullenix. Measurement of the effects of drugs on activity of permanent groups of rats. *Psychopharm. Commun.* **1:** 131–138, 1975.
72. Norton, S., P. Mullenix and B. Culver. Comparison of the structure of hyperactive behavior in rats after brain damage from x-irradiation, carbon monoxide and pallidal lesions. *Brain Res.* **116:** 49–67, 1976.
73. Norton, S. and W. Servos. Pattern recognition of rat behavior using a laboratory computer. 5th Int. Congress of Pharmacology, July 1972.
74. Peacock, L. J. and M. Williams. An ultrasonic device for recording activity. *Am. J. Psychol.* **75:** 648–652, 1962.
75. Peacock, L. J., M. H. Hodge and R. K. Thomas. Ultrasonic measurements and automatic analysis of general activity in the rat. *J. comp. physiol. Psychol.* **62:** 284–288, 1966.
76. Pirch, J. H. and R. H. Rech. Behavioral recovery in rats during chronic reserpine treatment. *Psychopharmacology* **12:** 115–122, 1968.
77. Post, R. M. and H. Rose. Increasing effects of repetitive cocaine administration in the rat. *Nature* **260:** 731–732, 1976.
78. Premack, D. Towards empirical behavioral laws: I. Positive reinforcement. *Psychol. Rev.* **66:** 219–233, 1959.
79. *Principles for Evaluating Chemicals in the Environment.* National Academy of Sciences, Washington, D.C., 1975.
80. Rafales, L. S., R. L. Bornschein, I. A. Michaelson, R. K. Loch and G. F. Barker. Drug induced activity in lead-exposed mice. *Pharmac. Biochem. Behav.* **10:** 95–104, 1979.
81. Rastogi, R. B. and R. L. Singhal. Lithium: Modification of behavioral activity and brain biogenic amines in developing hyperthyroid rats. *J. Pharmac. exp. Ther.* **201:** 92–102, 1977.
82. Rech, R. H. Antagonism of reserpine behavioral depression by *d*-amphetamine. *J. Pharmac. exp. Ther.* **146:** 369–376, 1964.
83. Rech, R. H., L. A. Carr and K. E. Moore. Behavioral effects of α-methyltyrosine after prior depletion of brain catecholamines. *J. Pharmac. exp. Ther.* **160:** 326–335, 1968.
84. Reed, J. D. Spontaneous activity of animals: A review of the literature since 1929. *Psychol. Bull.* **44:** 393–412, 1947.
85. Reiter, L. W., G. E. Anderson, J. W. Laskey and D. F. Cahill. Developmental and behavioral changes in the rat during chronic exposure to lead. *Envir. Hlth Perspect.* **12:** 119–123, 1975.
86. Reiter, L. W., K. Kidd, G. Ledbetter, L. Gray and N. Chernoff. Comparative behavioral toxicology of mirex and Kepone in the rat. *Toxic. appl. Pharmac.* **41:** 143, 1977.
87. Reiter, L. W. Behavioral toxicology: Effects of early postnatal exposure to neurotoxins on development of locomotor activity in the rat. *J. occup. Med.* **19:** 201–204, 1977.
88. Reiter, L. W. Use of activity measures in behavioral toxicology. *Envir. Hlth Perspect.* **26:** 9–20, 1978.
89. Remington, G. and H. Anisman. Genetic and ontogenetic variations in locomotor activity following treatment with scopolamine or *d*-amphetamine. *Devl. Psychobiol.* **9:** 579–585, 1976.
90. Richter, C. P. Animal behavior and internal drives. *Q. Rev. Biol.* **2:** 307–343, 1927.
91. Robbins, T. W. A critique of the methods available for the measurement of spontaneous motor activity. In: *Handbook of Psychopharmacology,* Vol. 7, edited by L. L. Iversen, S. D. Iversen and S. H. Snyder. New York: Plenum Press, 1977, pp. 37–82.

92. Rushton, R., H. Steinberg and C. Tinson. Effects of single experience on subsequent reaction to drugs. *Br. J. Pharmac.* **20:** 99–105, 1963.
93. Rushton, R., H. Steinberg and M. Tomkiewicz. Equivalence and persistence of the effects of psychoactive drugs and past experience. *Nature* **220:** 885–889, 1968.
94. Schaeffer, R. W. A new device for programming contingencies between drinking, running and level pressing. *J. exp. Analysis Behav.* **9:** 3–24, 1963.
95. Schnitzer, S. B. and S. Ross. Effects of physiological saline injections on locomotor activity in C57BL/6 mice. *Psychol. Rep.* **6:** 351–354, 1960.
96. Searle, L. V. and C. W. Brown. The effect of subcutaneous injections of benzedrine sulfate on the activity of white rats. *J. exp. Psychol.* **22:** 480–490, 1938.
97. Segal, D. S. Behavioral characterization of *d*- and *l*-amphetamine: Neurochemical implications. *Science* **190:** 475–477, 1975.
98. Segal, D. S. Behavioral and neurochemical correlates of repeated *d*-amphetamine administration. In: *Neurobiological Mechanisms of Adaptation and Behavior*, edited by A. J. Mandel. New York: Raven Press, 1975, pp. 247–262.
99. Segal, D. S. Differential effects of *para*-chlorophenylalanine on amphetamine-induced locomotion and stereotypy. *Brain Res.* **116:** 267–276, 1976.
100. Segal, D. S. and A. J. Mandell. Long-term administration of *d*-amphetamine: Progressive augmentation of motor activity and stereotypy. *Pharmac. Biochem. Behav.* **2:** 249–255, 1974.
101. Siegel, P. S. A simple electronic device for the measurement of gross bodily activity of small animals. *J. Psychol.* **21:** 227–236, 1946.
102. Silbergeld, E. K. and A. M. Goldberg. A lead-induced behavioral disorder. *Life Sci.* **13:** 1275–1283, 1973.
103. Silverman, A. P. and H. Williams. Behaviour of rats exposed to trichloroethylene vapour. *Br. J. Ind. Med.* **32:** 308–315, 1975.
104. Simpson, L. L. A study of the interaction between amphetamine and food deprivation. *Psychopharmacology* **38:** 279–286, 1974.
105. Skinner, B. F. *The Behavior of Organisms: An Experimental Analysis.* New York: Appleton-Century-Crofts, Inc., 1938, pp. 362–363.
106. Skinner, B. F. The measurement of spontaneous activity. *J. gen. Psychol.* **9:** 3–24, 1933.
107. Skinner, B. F. and W. H. Morse. Concurrent activity under fixed-interval reinforcement. *J. comp. physiol. Psychol.* **50:** 279–281, 1957.
108. Smith, C. B. Enhancement by reserpine and α-methyl dopa of the effects of *d*-amphetamine upon locomotor activity of mice. *J. Pharmac. exp. Ther.* **142:** 343–350, 1963.
109. Smith, C. B. and P. B. Dews. Antagonism of locomotor suppressant effects of reserpine in mice. *Psychopharmacology* **3:** 55–59, 1962.
110. Sobotka, T. J. and M. P. Cook. Postnatal lead acetate exposure in rats: Possible relationship to minimal brain dysfunction. *Am. J. Ment. Defic.* **79:** 5–9, 1974.
111. Steinberg, H., R. Rushton and C. Tinson. Modification of the effects of an amphetamine-barbiturate mixture by the past experience of rats. *Nature* **192:** 533–535, 1961.
112. Stewart, C. C. Variations in daily activity produced by alcohol and by changes in barometric pressure and diet, with a description of recording methods. *Am. J. Physiol.* **1:** 40–56, 1898.
113. Stewart, W. J. Environmental complexity does affect scopolamine induced changes in activity. *Neurosci. Let.* **1:** 121–125, 1975.
114. Stolk, J. M. and R. H. Rech. Enhanced stimulant effects of *d*-amphetamine on the spontaneous motor activity of rats treated with reserpine. *J. Pharmac. exp. Ther.* **158:** 140–149, 1967.
115. Stolk, J. M. and R. H. Rech. Enhanced stimulant effects of *d*-amphetamine in rats treated chronically with reserpine. *J. Pharmac. exp. Ther.* **163:** 75–83, 1968.
116. Stolk, J. M. and R. H. Rech. Antagonism of *d*-amphetamine by alpha-methyl-*l*-tyrosine. Behavioral evidence for the participation of catecholamine stores and synthesis in the amphetamine stimulant response. *Neuropharmacology* **9:** 249–263, 1970.
117. Strong, P. N. Activity in the white rat as a function of apparatus and hunger. *J. comp. physiol. Psychol.* **50:** 596–600, 1957.
118. Strong, P. N. and W. J. Jackson. Effects of hippocampal lesions in rats on three measures of activity. *J. comp. physiol. Psychol.* **70:** 60–65, 1970.
119. Su, M.-Q. and G. T. Okita. Behavioral effects on the progeny of mice treated with methylmercury. *Toxic. appl. Pharmac.* **38:** 195–205, 1976.
120. Sulser, F., M. L. Owens, N. R. Norvich and J. V. Dingell. The relative role of storage and synthesis of brain norepinephrine in the psychomotor stimulation evoked by amphetamine or by desipramine and tetrabenazine. *Psychopharmacology* **12:** 322–332, 1968.
121. Svensson, T. H. and G. Thieme. An investigation of a new instrument to measure motor activity of small animals. *Psychopharmacology* **14:** 157–163, 1969.
122. Tainter, M. L. Effects of certain analeptic drugs on spontaneous running activity in the white rat. *J. comp. Psychol.* **36:** 143–155, 1943.
123. Tapp, J. T., R. S. Zimmerman and P. S. D'Encarnacao. Intercorrelational analysis of some common measures of rat activity. *Psychol. Rep.* **23:** 1047–1050, 1968.
124. Tapp, J. T. Activity, reactivity and the behavior-directing properties of stimuli. In: *Reinforcement and Behavior*, edited by J. T. Tapp. New York: Academic Press, 1969, pp. 146–177.
125. Thornburg, J. E. and K. E. Moore. A comparison of effects of apomorphine and ET495 on locomotor activity and circling behavior in mice. *Neuropharmacology* **13:** 189–197, 1974.
126. Toman, J. E. P. and G. M. Everett. In: *Psychopharmacology*, edited by H. Pennes. New York: Hoeber-Harper, 1958, p. 248.
127. van Rossum, J. M. and F. Simons. Locomotor activity and anorexogenic action. *Psychopharmacology* **14:** 248–254, 1969.
128. Vasko, M. R., M. P. Lutz and E. F. Domino. Structure-activity relations of some indolealkylamines in comparison to phenethylamines on motor activity and acquisition of avoidance behavior. *Psychopharmacology* **36:** 49–58, 1974.
129. Villareal, J. E., M. Guzman and C. B. Smith. A comparison of the effects of *d*-amphetamine and morphine upon the locomotor activity of mice treated with drugs which alter brain catecholamine content. *J. Pharmac. exp. Ther.* **187:** 1–7, 1973.
130. Waldeck, B. Modification of caffeine-induced locomotor stimulation by a cholinergic mechanism. *J. Neural Transm.* **35:** 197–205, 1974.
131. Waldeck, B. On the interaction between caffeine and barbiturates with respect to locomotor activity and brain catecholamines. *Acta pharmac. toxic.* **36:** 172–180, 1975.
132. Walsh, R. N. and R. A. Cummins. The open-field test: A critical review. *Psychol. Bull.* **83:** 482–504, 1976.
133. Watzman, N., H. Barry, III, W. J. Kinnard, Jr. and J. P. Buckley. Comparison of different photobeam arrangements for measuring spontaneous activity in mice. *J. Pharmaceut. Sci.* **55:** 907–909, 1966.
134. Weasner, M. H., F. W. Finger and L. S. Reid. Activity changes under food deprivation as a function of recording device. *J. comp. physiol. Psychol.* **53:** 470–474, 1960.
135. Weissman, A., B. K. Koe and S. S. Tenen. Antiamphetamine effects following inhibition of tyrosine hydroxylase. *J. Pharmac. exp. Ther.* **151:** 339–352, 1966.
136. Wright, L. S., H. J. Horn, Jr. and G. Woodard. Activity patterns in mice tested singly and in groups as a drug screening tool. *Fedn. Proc.* **21:** 420, 1962.
137. Zabik, J. E., R. M. Levine and R. P. Maickel. Drug interactions with brain biogenic amines and the effects of amphetamine isomers on locomotor activity. *Pharmac. Biochem. Behav.* **8:** 429–435, 1978.
138. Zieve, L. Effect of benzedrine on activity. *Psychol. Rec.* **1:** 343–346, 1937.
139. Zucker, M. H. *Electronic Circuits for the Behavioral and Biomedical Sciences.* San Francisco: W. H. Freeman, 1969, Chapter 6.

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Reinforcing Properties of Inhaled Substances[1]

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WOOD, R. W. *Reinforcing properties of inhaled substances.* NEUROBEHAV. TOXICOL. **1:** Suppl. 1, 67–72, 1979.—Inhaled substances can support behavior by acting as reinforcing stimulus events. The deliberate inhalation of volatile materials is attributable to the positively reinforcing properties of these substances and can induce profound toxicity. On the other hand, inhaled substances can also be aversive, e.g., corrosives, certain solvents, and combustion products. Both positive and negative reinforcing properties of inhaled materials can be used to support the behavior of laboratory animals. Several rules of evidence should be met, however, to demonstrate conclusively that an inhalant has such properties. Such properties should be considered in industrial hygiene and environmental quality decisions.

Ammonia Sensory irritation Aversive atmosphere Escape behavior Mouse Irritants
Conditioning Behavior Toluene Nitrous oxide Self-administration Glue sniffing
Squirrel monkey Rhesus monkey Solvents Anesthetics Inhalant abuse

INHALED materials can affect behavior in a variety of ways, the most obvious of which is direct toxic impairment from acute or chronic exposure. Established techniques of behavioral pharmacology can evaluate such effects, but not without adequate attention to exposure techniques and pharmacokinetics. Inhaled materials also can alter behavior by acting as stimulus events. Such stimulus properties play a role in industrial hygiene and environmental quality decisions; laboratory techniques to study them have been reviewed [23]. This paper focuses on the positive and negative reinforcing stimulus properties of inhaled materials, will describe control procedures that can determine if materials have these properties, and will discuss their pertinence to regulatory decision making.

INHALANTS AS POSITIVE REINFORCERS

Volatile materials are subject to abuse by inhalation (sniffing). Deliberate inhalation of these materials is a menacing problem, especially among juveniles [18]. The materials subject to abuse encompass a wide variety of industrial and consumer products (Table 1; [2]), falling under the jurisdiction of several regulatory agencies. Establishing a uniform regulatory stance towards this substance abuse practice is much more complex than simply placing a new abused drug on a schedule of controlled substances.

The abuse of these materials is not limited to children; it also occurs on the job. As with any substance abuse practice, true incidence is difficult to assess, particularly inhalant abuse on the job, where discovery of the practice could lead to loss of employment. Access to the agents, of course, is a precondition to their abuse; some employees apparently regard access to these intoxicants as a job-related benefit. Occupational exposure to solvents has been associated with chronic abuse [6, 11, 16, 20]. Anesthesiologists and hospital technicians have been known to habitually self-administer halothane, cyclopropane, ether, nitrous oxide, ethyl chloride, and chloroform [12,19]. Even vinyl chloride has been subject to deliberate inhalation, with workers becoming like alcoholics [10].

TABLE 1

COMMERCIAL PRODUCTS SUBJECT TO ABUSE BY INHALATION (ADAPTED FROM [2])

Volatile Products	Aerosols
contact cement and adhesives	cold weather car starters
paints, lacquers and thinners	air sanitizers
dry cleaning fluids and spot removers	window cleaners
transmission and brake fluids	furniture polishes
liquid waxes and wax strippers	insecticides
shoe polishes	disinfectants
lighter fluids	spray medications
nail polish remover	deodorants and hair sprays
degreasers	antiperspirants
refrigerants	vegetable oil sprays
nitrites (amyl, butyl, isopentyl)	spray paints and lacquers
fuels	

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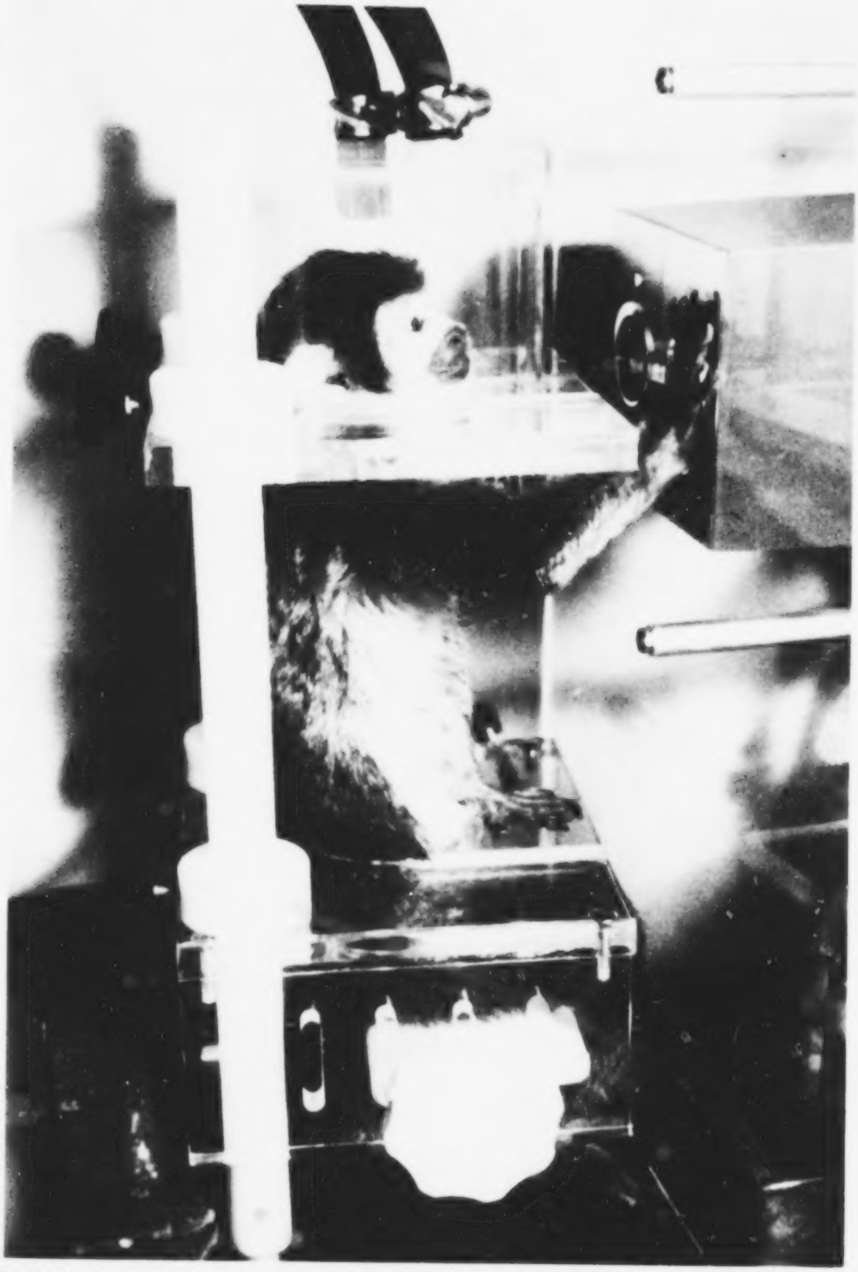

FIG. 1. Squirrel monkey seated in toluene self-administration apparatus. Subject is pushing button that produces a 15 sec infusion of toluene vapor into the helmet resting on the neck restraint plate of the chair.

It is important to be able to predict the abuse potential of substances incorporated in consumer products or that employees are likely to be exposed to transiently at relatively high concentrations. Examples are apparently innocuous solvents, refrigerants, propellants, and non-irritating volatile or gaseous substances. These materials may be toxic, or serve as a reinforcing vehicle for the ingestion of more toxic materials. Although non-human primates will self-administer most CNS drugs abused by humans [3, 9, 17], the positively reinforcing properties of inhaled substances have rarely been studied in the laboratory. Recently developed laboratory procedures demonstrate that volatile materials may act as

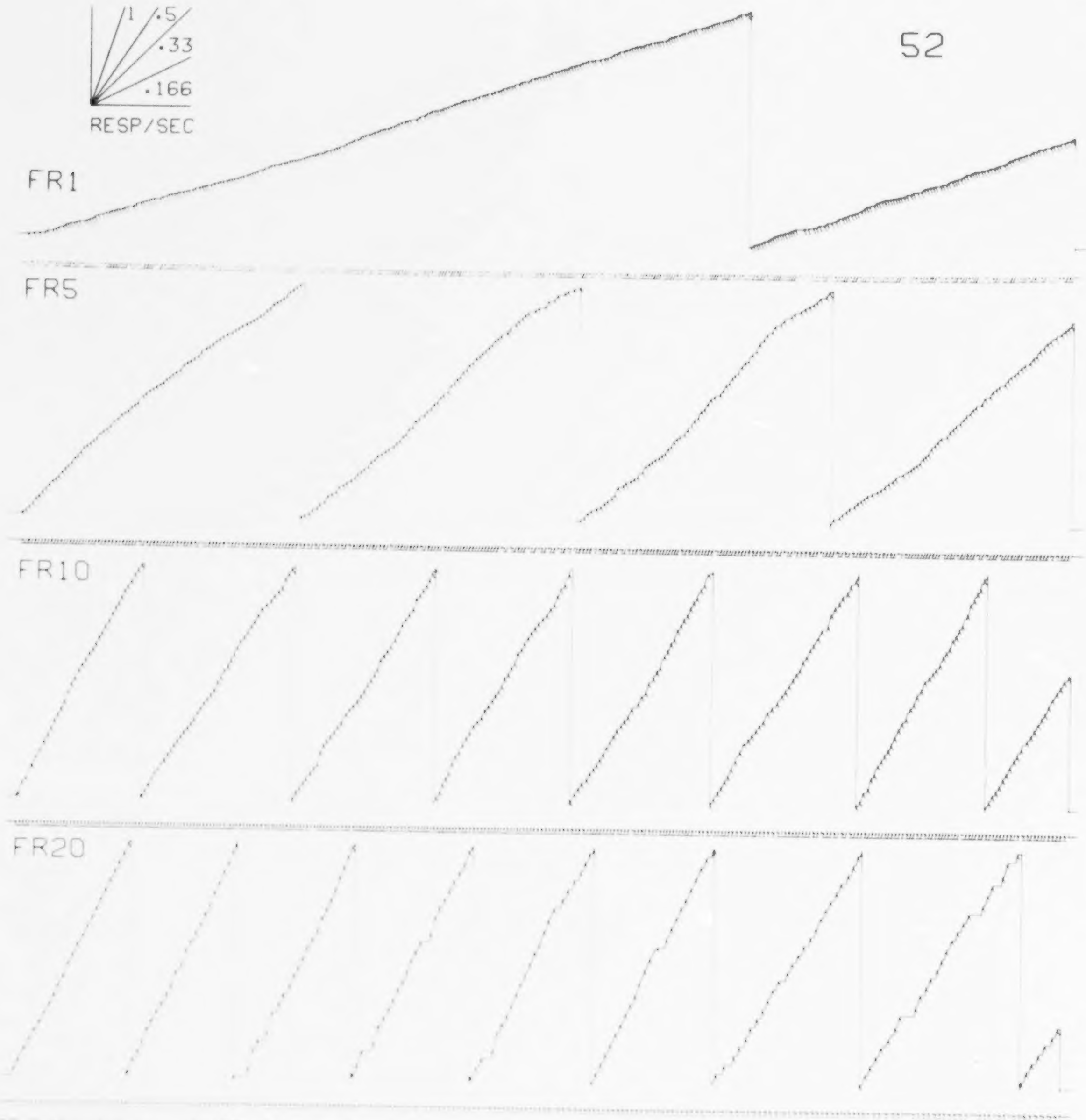

FIG. 2. Cumulative records of fixed-ratio performance maintained by 15 sec deliveries of 60% nitrous oxide for subject 52. Session duration was two hours. Increasing fixed-ratio size increased response rates. Deflection of stepping pen marks onset of reinforcement delivery; event pen is deflected for duration of reinforcement.

positive reinforcers, and bear substantial abuse potential. Several rules of evidence for such a demonstration are offered below.

An agent is a positive reinforcer if a response that produces the agent increases in frequency. This is implicit in any attempted demonstration and is subject to the qualifications listed below. Yanagita *et al.* [28] reported that macaque monkeys increased lever pressing when it produced intranasal infusions of chloroform, lacquer thinner, or ether. Wood *et al.* [26] demonstrated that squirrel monkeys will respond to produce delivery of nitrous oxide. Grubman [7] has demonstrated that the macaque also can be trained to self-administer nitrous oxide.

Terminating response-contingent access to the agent should eventually reduce the frequency of behavior (extinction). Ending nitrous oxide delivery lowered responding by 3 to 4 fold in squirrel monkeys [26]. Early in the monkeys' self-administration history, withholding toluene vapor produced irregular responding that occurred in bursts [23,24]. The toluene self-administration preparation is shown in Fig. 1. Oxygen or nitrogen substitution for nitrous oxide did not support the self-administration behavior of macaques [7].

Typical patterns of behavior should be generated by schedules of reinforcement. Increasing the number of responses required for reinforcement increases response rate with a variety of reinforcers, such as food [1] and cocaine [4, 5, 13]. This is also the case with nitrous oxide in the squirrel monkey (see Fig. 2: [26]) and in the macaque [7]. Rhesus monkeys also display typical patterns of responding with a multiple fixed-ratio 30 fixed-interval 5 min schedule of reinforcement [7].

The frequency of responding maintained by the agent should be a function of concentration. Response and reinforcement rates usually are related by an inverted U-shaped function to the magnitude of the event maintaining behavior. Examples are provided by heat in the cold [21], electrical stimulation of the brain [14,15], food [4], and a wide variety of drugs injected intravenously [4, 8, 15, 22, 27]. This is also the case with toluene self-administration by the squirrel monkey [23,24] and nitrous oxide self-administration by the macaque [7]. This relationship was not cleanly demonstrated in squirrel monkeys that self-administered nitrous oxide [26]. Beyond the minimum concentration necessary to maintain the behavior, the relationship between nitrous oxide concentration and reinforcement rate was flat (15–75%). However, when 20 responses were required for nitrous oxide delivery, response and reinforcement rates were an increasing function of concentration. Within the concentrations employed, rate decrements were not produced by increasing the nitrous oxide concentration, with the exception of one transition in one subject.

The behavior must not result from a general rate-increasing effect of the agent. Squirrel monkeys that self-administered toluene were seated in a chair facing two buttons [23,24]. A response on one button diverted a portion of the air stream through toluene in a gas washing bottle and back into the helmet (Fig. 1). Responses on the second button had no effect. Animals responded at a higher rate on the active button, and was not related to concentration, indicating that the performance did not result from a general inreversed. Responding on the active button was related to concentration, as described above. Responding on the inactive button occurred at a much lower rate than on the active button, and was not related to concentration indicating that the performance did not result from a general increase in response rate produced by toluene. Noncontingent toluene delivery also reduced the frequency of self-administration, further demonstrating that responding is maintained by the reinforcing properties of toluene, and is not merely a nonspecific increase in responding.

INHALANTS AS NEGATIVE REINFORCERS

Air pollutants and other inhaled materials can display noxious biological effects ranging from unpleasant odor and eye and upper airway irritation, to tissue destruction. Laboratory studies of inhalants that examine physiological or morphological changes address the behavioral significance of exposure only indirectly. The aversiveness of an inhalant stimulus could be assessed by determining the concentrations at which an animal responds to turn it off. If the stimulus is intense enough so that its termination supports behavior, then it is an effective negative reinforcer. A tech-

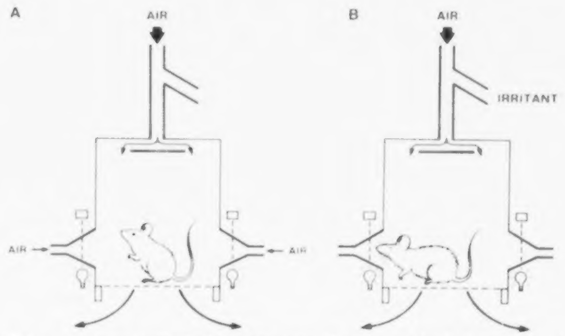

FIG. 3. Irritant (ammonia) exposure chamber: (A) before irritant delivery; (B) during irritant delivery. The chamber atmosphere was introduced from the top of the chamber, where it struck a baffle to ensure even mixing. The mouse stood on a perforated stainless steel platform through which the atmosphere exhausted. The irritant was added to the dilution air immediately above the chamber. The irritant delivery could be terminated by the mouse interrupting a light beam located in a conical recess in the wall. At any given time, only one of these two sensors would terminate the irritant delivery. When the irritant was shut off, either by a nose poke or at the end of 60 sec, a 1 liter/min stream of clean humidified air was delivered through each cone. This was done to minimize the delay of irritant termination after a response occurred.

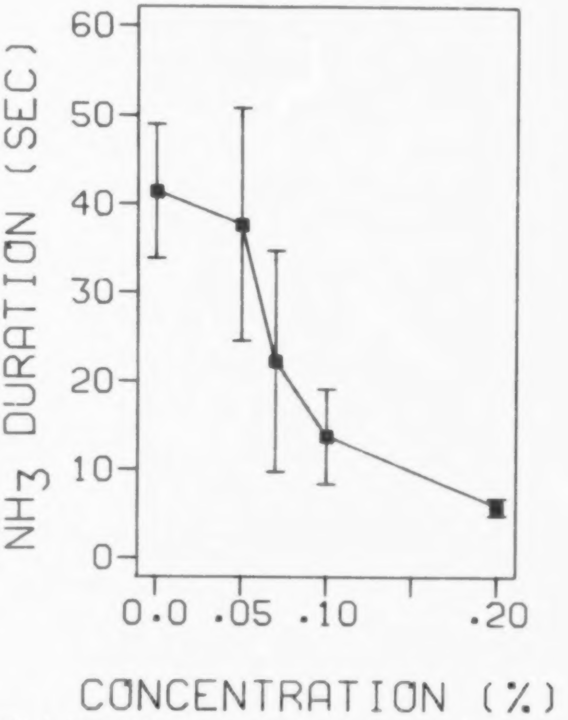

FIG. 4. Average duration of ammonia delivery as a function of ammonia concentration. These data represent the mean performance of six mice given one session of 50 deliveries at each concentration, except for the 0.1% level, which is the mean of the means of 4–10 sessions per mouse. Plotted are the means ± 1 SD.

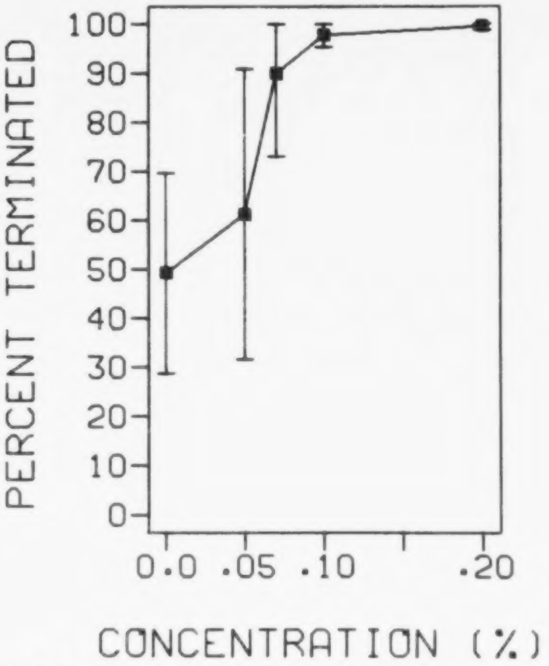

FIG. 5. Percentage of ammonia deliveries terminated by a response as a function of ammonia concentration. Data are as described in Fig. 4.

nique has recently been developed to investigate the determinants of escape from an aversive atmosphere [25]. It is illustrated in Fig. 3. Work done by this technique can serve to illustrate the control procedures that must be performed in order to demonstrate conclusively escape from aversive atmospheres. They include the following:

An inhalant is a negative reinforcer if the duration of exposure tolerated is inversely related to concentration. When mice were given the opportunity to terminate ammonia delivery, they tolerated a progressively shorter duration of ammonia as concentration increased (Fig. 4).

An inhalant is a negative reinforcer if the proportion of deliveries terminated by a response is directly related to concentration. The mice terminated a greater percentage of the ammonia deliveries as the concentration increased to 0.1%; at this and a higher concentration (0.2%) virtually every delivery was terminated by the mice (Fig. 5).

The behavior must not result from a general rate-increasing effect of the agent. Responses per delivery cycle (irritant onset to onset) were related to concentration on the sensor that terminated delivery, while responses per cycle on the inactive sensor were not so related. If the nose-poking behavior in this preparation was generated by a general increase in activity, a differential frequency of responding between the two identical sensors would not be expected.

The subject must discriminate between responses that terminate inhalant delivery and those that do not. On approximately 85% of the trials terminated by a response, the animals went directly to the appropriate sensor without making a response on the inappropriate sensor. When the function of the two sensors was exchanged, the mice were behaving appropriately by the third hour after the reversal.

APPLICATIONS AND IMPLICATIONS

Preparations that evaluate inhalants as positive or negative reinforcing stimuli address a variety of questions pertinent to regulatory decision making. Both classes of procedures help determine the irritant potency and abuse potential of materials. Time- or exposure-dependent changes arising from adaptation, sensitization, or tolerance can also be determined. Adaptation phenomena are of special importance for aversive inhalants since the unpleasant properties of materials are sometimes relied upon to limit worker exposure in industrial settings. Many compounds could be studied using such techniques, including corrosives, solvents, and the combustion products of industry, the automobile, and the catastrophic conflagration.

Intoxicating substances pose a special hazard to workers who develop a fondness for the materials with which they work. These properties should be taken into account when short-term exposure limit values are set. The abuse potential of inhalants can be assessed without resorting to experimental human exposures. In addition, animal self-administration preparations should be able to determine self-administration limit values, i.e., exposure levels insufficient to maintain the abuse of these materials. It should be remembered that the exposures generated during the abuse of these materials are usually high concentration spikes, and that these exposures are unlike those used in typical evaluations of toxicity. The consistency and chronicity of these spiked exposures can be remarkable; for example, one man noticed that toluene produced euphoria while working with pain thinner at an aircraft company. His habit continued over a fourteen-year period, and escalated to deep inhalation of concentrated vapors more than ten times per hour throughout the day, including mealtimes [11]. The reinforcing properties of these materials are critical when these materials are carcinogenic, have acute organ toxicity, or induce chronic toxicity such as polyneuropathies.

REFERENCES

1. Boren, J. J. Resistance to extinction as a function of the fixed ratio. *J. exp. Psychol.* **61:** 304–308, 1961.
2. Cohen, S. Inhalant abuse: an overview of the problem. In: *Review of Inhalants: Euphoria to Dysfunction*, edited by C. W. Sharp and M. L. Brehm. NIDA Research Monograph 15, USDHEW Publication No. (ADM) 77-55 pp. 2–11, 1977.
3. Deneau, G. A., T. Yanagita and M. H. Seevers. Self-administration of psychoactive substances by the monkey—a measure of psychological dependence. *Psychopharmacologia* **16:** 30–48, 1969.
4. Goldberg, S. R. Comparable behavior maintained under fixed-ratio and second-order schedules of food presentation, cocaine injection or d-amphetamine injection in the squirrel monkey. *J. Pharmac. exp. Ther.* **186:** 18–30, 1973.
5. Goldberg, S. R., F. Hoffmeister, U. U. Schlichting and W. Wuttke. A comparison of pentobarbital and cocaine self-administration in rhesus monkeys: effects of dose and fixed-ratio parameter. *J. Pharmac. exp. Ther.* **179:** 277–283, 1971.
6. Grabsky, D. A. Toluene sniffing producing cerebellar degeneration. *Am. J. Psychiat.* **118:** 461, 1961.

7. Grubman, J. Behavior maintained by nitrous oxide reinforcement in the rhesus monkey. Unpublished masters thesis, Department of Pharmacology, University of Michigan, 1977.
8. Hoffmeister, F. and U. U. Schlichting. Reinforcing properties of some opiates and opioids in rhesus monkeys with histories of cocaine and codeine self-administration. *Psychopharmacologia* **23:** 55–74, 1972.
9. Kelleher, R. T., S. R. Goldberg and N. A. Krasnegor. Symposium on control of drug-taking behavior by schedules of reinforcement. *Pharmac. Rev.* **27:** 287–555, 1975.
10. Klein, J. The plastic coffin of Charlie Arthur. Rolling Stone, pp. 43–47, January 15, 1976.
11. Knox, J. M. and J. R. Nelson. Permanent encephalopathy from toluene inhalation. *New Engl. J. Med.* **275:** 1494–1496, 1966.
12. Nagle, D. R. Anesthetic addiction and drunkeness: a contemporary and historical survey. *Int. J. Addict.* **3:** 25–39, 1968.
13. Pickens, R. and T. Thompson. Cocaine-reinforced behavior in rats: effects of reinforcement magnitude and fixed ratio size. *J. Pharmac. exp. Ther.* **161:** 122–129, 1968.
14. Plutchik, R., W. L. McFarland and B. W. Robinson. Relationships between current intensity, self-stimulation rates, escape latencies, and evoked behavior in rhesus monkeys. *J. comp. physiol. Psychol.* **61:** 181–188, 1966.
15. Reynolds, R. W. The relationship between stimulation voltage and rate of hypothalamic self-stimulation in the rat. *J. comp. physiol. Psychol.* **51:** 193–198, 1958.
16. Satran, R. and V. N. Dodson. Toluene habituation: report of a case. *New Engl. J. Med.* **268:** 719–721, 1963.
17. Schuster, C. R. and T. Thompson. Self-administration of and behavioral dependence on drugs. *Ann. Rev. Pharmac.* **9:** 483–502, 1969.
18. Sharp, C. W. and M. L. Brehm. *Review of Inhalants: Euphoria to Dysfunction.* NIDA Research Monograph 15, U.S.D.H.E.W. Publication No. (ADM) 77-553, 1977.
19. Spencer, J. D., F. O. Raasch and F. A. Trefny. Halothane abuse in hospital personnel. *J. Am. Med. Ass.* **235:** 1034–1035, 1976.
20. Weisenberger, B. L. Toluene habituation. *J. occup. Med.* **19:** 569–570, 1977.
21. Weiss, B. and V. G. Laties. Magnitude of reinforcement as a variable in thermoregulatory behavior. *J. comp. physiol. Psychol.* **53:** 603–608, 1960.
22. Wilson, M. C., M. Hitomi and C. R. Schuster. Psychomotor stimulant self-administration as a function of dosage per injection in the rhesus monkey. *Psychopharmacologia* **22:** 271–281, 1971.
23. Wood, R. W. Stimulus properties of inhaled substances. *Envir. Hlth. Perspect.* **26:** 69–76, 1978.
24. Wood, R. W. Toluene self-administration by the squirrel monkey. *Seventh Int. Cong. Pharmac.*, Paris, 1978, Abstract 1497.
25. Wood, R. W. Behavioral evaluation of sensory irritation evoked by ammonia. *Toxicol. Appl. Pharmac.*, in press, 1979.
26. Wood, R. W., J. Grubman and B. Weiss. Nitrous oxide self-administration by the squirrel monkey. *J. Pharmac. exp. Ther.* **202:** 491–499, 1977.
27. Woods, J. H. and C. R. Schuster. Reinforcement properties of morphine, cocaine and SPA as a function of unit dose. *Int. J. Addict.* **3:** 231–237, 1968.
28. Yanagita, T., S. Takahashi, K. Ishida and H. Funamoto. Voluntary inhalation of volatile anesthetics and organic solvents by monkeys. *Jap. J. clin. Pharmac.* **1:** 13–16, 1970.

Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function
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Behavioral Assessment of Risk-Taking and Psychophysical Functions in the Baboon

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BRADY, J. V., L. D. BRADFORD AND R. D. HIENZ. *Behavioral assessment of risk-taking and psychophysical functions in the baboon.* NEUROBEHAV. TOXICOL. **1:** Suppl. 1, 73–84, 1979.—Laboratory procedures have been developed for the experimental analysis of risk-taking and psychophysical functions in dog-faced baboons (*Papio anubis*). In a procedure analogous to the traffic light situation, animals are rewarded with food pellets for completing a fixed ratio of 100 responses in the presence of a green light. Superimposed upon this baseline performance are 5-second presentations of a yellow warning light terminated by a red light in the presence of which all responses are punished with electric shock. When the yellow light is introduced late in the sequence (e.g., after 93 responses have been completed), response rates increase and the 100-response ratio is completed before the 5-second yellow light times out. When the yellow light appears early in the sequence (e.g., after 73 responses) a marked decrease in response rate is observed with cessation of responding before onset of the red light. The sensitivity of components of this risk-taking performance to pharmacological toxicants is reported and psychophysical assessment of relevant sensory-motor effects described.

Risk-taking Psychophysical functions Baboons Pentobarbital Diazepam d-Methylamphetamine Chlordiazepoxide Visual thresholds Auditory thresholds Reaction time Pharmacological toxicants Behavioral toxicology Amobarbital

THE DEVELOPMENT of test methods for defining the effects of toxic substances on behavior have for the most part focused upon well-explored procedures of demonstrated sensitivity, usually with pharmacological or physiological parametric interactions as a reference base [7]. The scope of such methodological approaches has included a broad range of naturalistic (e.g., activity and feeding cycles) and learned (e.g., schedule-controlled performances) behaviors [4], as well as innovative and sophisticated combinations of the two in the application of psychophysical procedures to basic sensory [2,5] and motor [8] assessments. Characteristically, these developments have reflected a shift from traditional screening approaches involving limited observations on large numbers of organisms to more precise assessment techniques where the large N is provided by the number of observations (frequently of more than one behavior simultaneously) and the number of organisms is relatively small. The obvious advantages of such precision evaluations include the ability to focus upon specific behavioral systems under well-controlled baseline conditions which minimize the likelihood that minor random perturbations will contribute significantly to the observed variance.

The extension of this behavioral toxicology assessment approach to less well-analyzed affective repertoires would seem to require a comparable shift from dependence upon traditionally subjective psychological evaluations to performance measures derived from applied behavior analysis techniques [6]. Furthermore, experimental developments emphasizing the analysis of such complex behavioral interactions and psychophysical processes at the animal laboratory level should serve to counteract the tendency for performance assessment methods and measures in behavioral toxicology to remain rigid in response to the dictates of familiarity. In this regard, recent efforts to extend the breadth and sensitivity of animal behavior baselines for evaluating the effects of pharmacological toxicants have explored experimental procedures for the analysis of risk-taking performances in laboratory baboons [1].

One such procedure may be described as an analogue of the traffic light situation commonly encountered in urban ecologies. In the presence of a green light, the animal is rewarded with food pellets for completing a fixed number of responses (e.g., 100) on a spring-loaded lever switch (i.e., Lindsley manipulandum). An added counter is provided for the animal in the form of 10 small white pilot lights illuminated sequentially upon completion of each block of 10 responses, thus serving to indicate location and progression through the 100-response ratio requirement. Superimposed upon this baseline performance are presentations of a yellow light stimulus (5 sec in duration) which replaces the green light and can occur at any point in the fixed 100-response ratio sequence. The 5-sec yellow light interval is terminated by the appearance of a red light (60 sec in duration) in the presence of which all lever responses are punished by an electric shock administered through an electrode attached to a shaved portion of the baboon's tail. Responses in the presence of both the yellow and red light serve to advance the 100-response count required for delivery of the food pellet reward even though each response in the red light is accompanied by shock.

Initial explorations of this procedure have focused upon the effects of the yellow warning light, with the not unex-

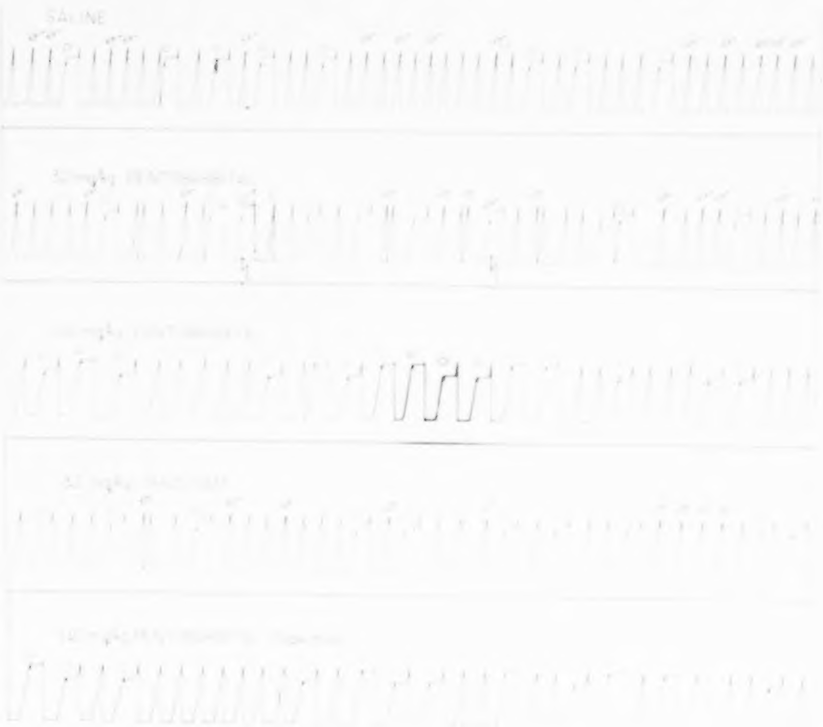

FIG. 1. Cumulative records illustrating baseline risk-taking performance and selective drug effects for Baboon Q-74.

pected finding that response rates under such conditions differ as a function of the location of the 5-sec yellow light interval in the 100-response ratio sequence. When the warning light is introduced late in the sequence (e.g., after 90 of the 100 required responses have been completed), the rate in yellow is increased above the 2–3 responses/sec rate observed during comparable intervals in the presence of the green light alone. Under such circumstances, the 100-response ratio is completed well before the 5-sec yellow light times out and before the red light appears. In contrast, introduction of the yellow light early in the sequence (e.g., after completion of only 60 of the required 100 responses) produces a marked decrease in the yellow response rate, with cessation of responding occurring well before onset of the red light. Under such conditions, suppression continues throughout the 60-sec red light interval and responding resumes upon its termination (i.e., reappearance of the green light). Although it has not as yet been possible to reliably determine risk threshold values (e.g., the point in the required 100-response ratio sequence at which introduction of the yellow light results in rate increases 50% of the time and rate decreases 50% of the time), a relatively stable performance baseline has been developed and its potential sensitivity as a behavioral assessment procedure of pharmacological and/or toxicological relevance is presently being evaluated.

Figure 1, for example, illustrates the performance baseline presently being used for assessing the effects of pharmacological toxicants on risk-taking behaviors. In the course of daily 3–4 hour experimental sessions, three different types of trials recur randomly (and in equal numbers), representing different levels of behavioral risk. Baseline no-risk trials consist of green light presentations alone without yellow or red light intrusions to interrupt the 100-response sequence which produces food. Low-risk trials occur when the yellow light is presented late in the 100-response sequence (i.e., after the 93rd response) and the ratio required for food delivery is easily completed (i.e., at response rates well above 2–3 per sec) before 5 sec elapse and the red light appears. And the high risk trials are defined by presentation of the yellow light relatively early in the 100-response sequence (i.e., after the 73rd sequence) under conditions which make it all but impossible for the animal to complete the remaining 27 ratio responses required for food reward in the 5 sec before appearance of the red light and response-contingent shock.

The record shown at the top of Fig. 1 illustrates the stable performance baseline which has developed under these multiple risk schedule conditions with Baboon Q-74. The low risk trials during which yellow light presentations occurred late in the required 100-response sequence are marked with a symbol (↗) signifying that in each instance the yellow light was run and the animal completed the ratio and obtained food before 5 sec elapsed and the red light appeared. In contrast, the high risk trials marked by the symbol, during which yellow light presentations occurred early in the required 100-response sequence, were uniformly characterized by a slowing of the response rate in the presence of the yellow light followed by cessation of responding both before and during the red light interval. The remaining green light alone trials (with no symbol marking superimposed) on the

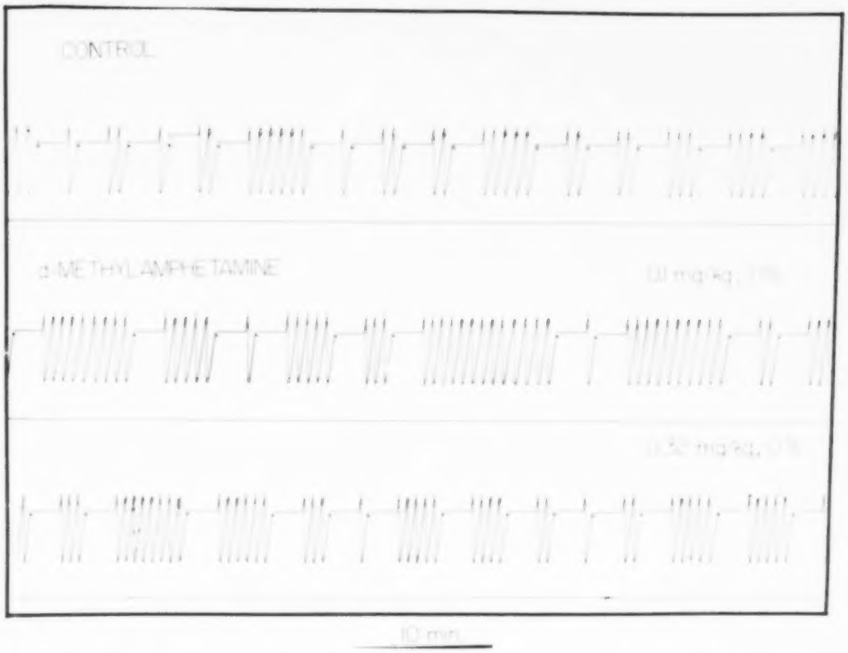

FIG. 2. Baseline control performance (top record) and portions of two drug sessions following doses of 0.1 mg/kg (middle record) and 0.32 mg/kg (bottom record) *d*-methylamphetamine for Baboon S-WI under low shock risk (0% shock probability) conditions.

other hand, can be seen to have produced a constant rate of responding throughout the required 100-response sequence.

Of particular interest, however, would seem to be the potentially selective sensitivities in this multiple risk-taking performance baseline suggested by the drug effects illustrated in several cumulative records shown in Fig. 1. Pentobarbital (10 mg/kg), for example, administered intramuscularly between 1 and 2 hr prior to the performance sample shown in the third record from the top can be seen to have differentially suppressed virtually all responding in the presence of the yellow lights, and in effect, eliminated even the low-risk runs, as indicated by the symbols ( ) above each such trial. And this differential effect occurred (and was replicated, as shown in the bottom record of Fig. 1) with only a relatively modest decrease in response rates during green light intervals. The dose-dependent nature of this effect is also indicated by the second record from the top in Fig. 1, which shows that 3.2 mg/kg pentobarbital had no such suppressing effect upon the yellow rate, although on at least two occasions at this dose, the animal made unprecedented high risk runs into the red light and received response-contingent shock punishment (indicated by the broken arrows on the base of the record). Finally, the second record from the bottom in Fig. 1 shows that at least an initial dose of 3.2 mg/kg diazepam produced no such effect upon the risk-taking performance with this animal, though some slowing of the overall response rate was apparent.

The effects of *d*-methylamphetamine on this performance baseline was also studied, and an additional dimension of risk-taking was investigated. A variation in the basic procedure with a second animal (S-WI) provided for changes in the probability that responses in the presence of the red light produced shock. Under conditions of high shock risk, 100% of the responses in red were shocked (i.e., the procedure illustrated in Fig. 1); alternatively, in the low shock risk condition, 0% of the responses in red produced shock. The two conditions were programmed in mixed order over successive experimental days with drug injections and interspersed vehicle control sessions. Figure 2 shows the baseline control performance (top record) and portions of two drug sessions following doses of 0.1 mg/kg (middle record) and 0.32 mg/kg (bottom record) *d*-methylamphetamine for baboon S-WI under low shock risk conditions with a red light duration of 120 sec. Despite the modest rate-increasing effects of the drug, no change in the risk-taking performance occurred with either this animal (S-WI) or the animal whose records are shown in Fig. 1 (Q-74) as a consequence of *d*-methylamphetamine administration. Significantly, the risk-taking performances were unchanged under such conditions even though shock probabilities were reduced to zero (e.g., middle and bottom sections of Fig. 2) for baboon S-WI.

When the experiments with pentobarbital illustrated in Fig. 1 for baboon Q-74 were systematically replicated with baboon S-WI under conditions involving variations in shock probability, however, an additional dimension of drug effects was observed. The top section of Fig. 3, for example, shows the performance of S-WI on the risk-taking procedure with 0% shock probability after 5.6 mg/kg pentobarbital. Virtually all discrimination between early and late yellow light presentations was lost, and despite the moderately suppressing effect of the drug on overall response rate, a dramatic increase in responding during both the early yellow and red light intervals was accompanied by a high incidence of earned (but not delivered) punishment shocks (event

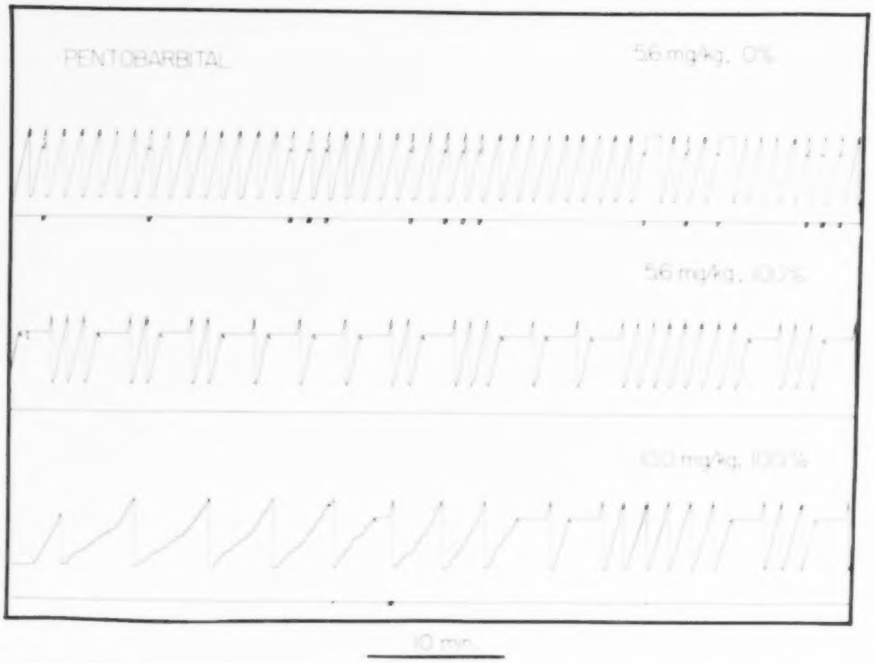

FIG. 3. Cumulative records showing effects of pentobarbital (5.6 and 10.0 mg/kg) upon performance under conditions of low shock risk (0% shock probability, top record) and high shock risk (100% shock probability, middle and bottom records) for Baboon S-WI.

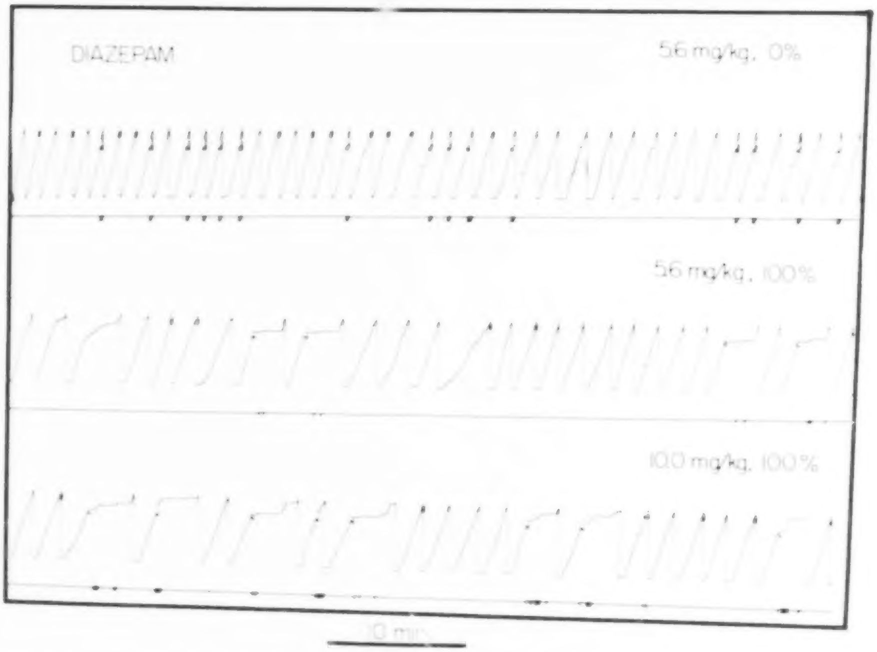

FIG. 4. Cumulative records showing effects of diazepam (5.6 and 10.0 mg/kg) upon performance under conditions of low shock risk (0% shock probability, top record) and high shock risk (100% shock probability, middle and bottom records) for Baboon S-WI.

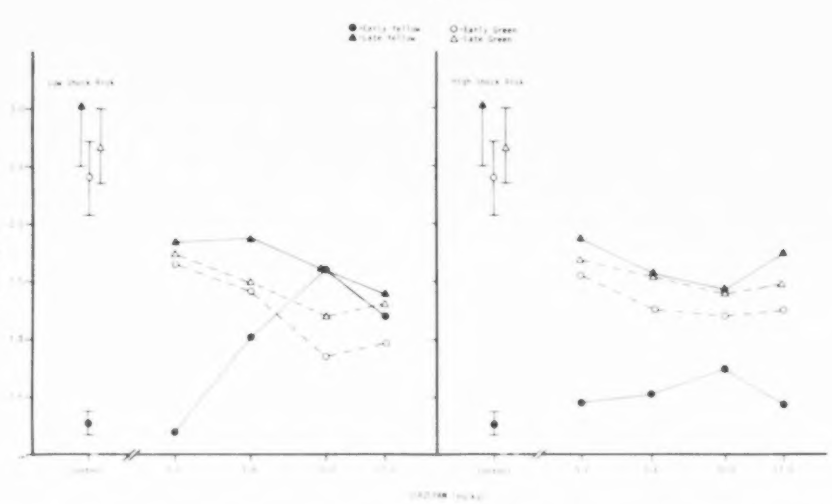

FIG. 5. The effects of diazepam upon "risk taking" behavior and control rates under conditions of low shock risk (0% shock probability, left panel) and high shock risk (100% shock probability, right panel) for Baboon S-WI. Brackets enclosing control values indicate ± one standard deviation.

marks on baseline under top record). With the same dose of pentobarbital (5.6 mg/kg) under the 100% shock probability condition, however, this same animal (S-WI) generated the performance shown in the middle record of Fig. 3. Except for the moderate overall rate decrease, the 5.6 mg/kg dose of pentobarbital under these conditions had little apparent effect upon risk-taking, and there was a striking similarity to the baseline performance for this animal (i.e., S-WI) shown in the top section of Fig. 2. With the higher dose of pentobarbital (i.e., 10.0 mg/kg) however, shown in the bottom record of Fig. 3, rather severe rate suppression and some disruption of the risk-taking performance were apparent even with the 100% shock probability condition in effect.

Figure 4 shows a similar progression of experiments with diazepam for baboon S-WI under conditions of low and high shock probability. Again, a marked increase in responding during both the early yellow and red light intervals accompanied by a high incidence of earned but undelivered shocks followed administration of 5.6 mg/kg diazepam under the 0% shock probability condition (top record, Fig. 4) despite evident decreases in the overall response rate. And although some normalization of the risk-taking performance was apparent when the same diazepam dose (i.e., 5.6 mg/kg) preceded an experimental session programmed under the 100% shock probability condition (middle record, Fig. 4), intermittent disruptions (e.g., running through the early yellow-light; responses initiated in the presence of the red light) continued to occur and were observed with even higher frequency following 10.0 mg/kg diazepam (bottom record, Fig. 4). In this regard, the apparent greater sensitivity of baboon S-WI (i.e., by comparison with baboon Q-74) to pentobarbital and diazepam is reflected in both the overall rate and the risk-taking performance changes following drug administration.

The extent to which these drug-related performance changes can be characterized as effects upon risk-taking depends, of course, upon a critical analysis of differential sensitivity to such pharmacological toxicants of selected components of the multi-operant repertoire shown in Figs. 1 through 4. Figure 5, for example, illustrates such a component analysis and compares the effects of diazepam over the dose range of 3.2 to 17.0 mg/kg under conditions of low (i.e., 0% probability) and high (i.e., 100% probability) shock risk. The right side of Fig. 5 shows the generally suppressing effect of diazepam at all four doses upon response rates in the presence of the green light and the late (i.e., after the 93rd response in the 100-response green light sequence) yellow light while at the same time producing at least a moderate increase in the early (i.e., after the 73rd response in the 100-response green light sequence) yellow response rate (solid line connecting filled circles at bottom of graph). The data points plotted as open circles and triangles represent control response rates measured during green-light-only trials over early and late time segments in the 100-response sequence corresponding to the exact location (i.e., after the 73rd and 93rd responses, respectively) of those intervals during yellow light trials. The orderly and systematic relationships between these rates and those measured in the presence of the early and late yellow light (i.e., filled circles and triangles, respectively) clearly differentiate the component performances of this multi-operant risk-taking repertoire. The late yellow rates (filled triangles) are slightly but consistently higher than the late green rates (open triangles), while the early yellow rates (filled circles) are consistently and markedly lower than the early green rates (open circles). And at least under the high shock risk condition (right side, Fig. 5), these relationships are as stable across all indicated doses of diazepam as they are during the no-drug control sessions from which the data shown in the upper left hand corner of each graph were derived.

Though the differential (and somewhat dose-dependent) increase in early yellow response rates following diazepam administration under the high shock risk condition shown on the right side of Fig. 5 is suggestive of a pharmacological toxicant effect upon selective aspects of risk-taking, the well-established rate-dependency hypothesis (i.e., a wide range of drugs are known to decrease high rates of responding and increase low rates) would seem to provide a more parsimonious interpretive alternative. Such is not the case, however, when the data from the low shock risk condition shown on the left side of Fig. 5 is considered. Again, all four doses of diazepam can be seen to suppress response rates in the presence of the green light and the late yellow light. But the dramatic (and clearly dose-dependent) increase in responding during the early yellow light (solid line connecting filled circles) under this low shock risk condition (i.e., 0% shock probability) can not be attributed to a drug effect upon low response rates alone. Both the low and high shock risk condition are characterized by equally low response rates in the early yellow under control conditions. But only in the low shock risk conditions did diazepam increase the early yellow rate above the early and late green rates. It would appear that diazepam has differential effects upon risk-taking performances depending upon whether there is a high or low shock risk.

There remain, of course, many unanswered questions about the validity, reliability, selectivity, feasibility, and economics of such an experimental approach to defining the effects of toxic substances upon so-called risk-taking behaviors. Not the least among these concerns is the extent to which effects rather glibly described in such interpretive language can be accounted for in terms of the sensory and motor processes upon which the performances involved obviously have a basic dependence. To help define some of these conceptual and methodological limits, a series of studies was undertaken to assess the sensory and motor effects of those pharmacologic toxicants which appeared to alter risk-taking. The psychophysical methodology [3] involved the use of a reaction-time procedure which required the baboons to press a lever and hold it depressed for varying intervals until presentation of a light flash or tone burst signalled the availability of food reward following lever release. Correct responses (i.e., lever releases occurring within 1.5 sec of signal onset) were rewarded with banana-flavored food pellets, and detection thresholds were determined by systematically varying stimulus intensity and recording the frequency of correct and incorrect responses (i.e., lever releases occurring later than 1.5 sec after stimulus onset). In addition, response latencies (i.e., elapsed time between signal onset and lever release) were recorded to the nearest millisecond as a measure of reaction time.

The subjects in the studies completed to date were 4 dog-faced baboons (*Papio anubis*), housed in individual cages and maintained on a 22-hr restricted feeding schedule with supplemental monkey chow and fresh fruit provided on a daily basis after each experimental session. The testing apparatus consisted of a modified baboon squeeze cage fitted within a double-walled sound attenuating chamber. A 30 × 38 inch intelligence panel attached to one side of the cage contained a microswitch actuating lever, a red LED cue light, a one-inch circular visual stimulus patch, and a tube feeder for delivery of banana pellets. With the animal positioned facing the panel, the cue light and visual stimulus patch were at eye level, the feeding tube at mouth level, and the response lever at waist level in front of the right arm. Additionally, a wide-range acoustic driver suspended outside the cage and located directly over the animal's head approximately 8 inches above ear level provided for the delivery of auditory signals.

The light source for the visual stimuli was provided by a slide projector mounted on the outside of the chamber and projecting white light on to the back of the one-inch stimulus patch through an otherwise light-tight aperture in the chamber wall. Stimulus intensity was varied by using neutral density filters in the slide projector. Light intensities were calibrated with a light meter. Acoustic signals were generated by a Krohn-Hite oscillator passed through an electronic switch (20 msec rise and fall times), programmable attenuator, amplifier, and the wide range acoustic drive (i.e., speaker). The system was calibrated with a General Radio sound level meter, and a Bruel and Kjaer amplifier, and ½ inch condenser microphone located at ear level facing the speaker. Programming of the experiments was accomplished with a solid-state control system. Data recording involved the use of electromechanical counters and a microprocessor interfaced to a video terminal which recorded all response latencies and computed median latency and Q values.

Following initial shaping of lever pressing and discrimination of the holding and release components of the response, all animals were introduced to the discrete trial reaction time procedure. In the presence of a flashing red cue light (5/sec), a lever press changed the flashing red light to a steady red light which remained instated as feedback as long as the animal held the lever switch in the closed position. At varying intervals (range 0.5 to 6.5 sec) following initiation of this maintained holding response, a test stimulus (white light on the circular patch or tone burst through the speaker) was presented for 1.5 sec. Release of the lever within the 1.5 sec test stimulus interval delivered a single banana pellet and initiated a 1 sec intertrial interval (ITI) during which no stimuli were presented and lever responses re-initiated the ITI. Incorrect responses (i.e., lever presses prior to test stimulus onset or after the 1.5 sec test stimulus interval) reinstated the 1 sec ITI without reinforcement. Following the 1 sec ITI, the flashing red cue light signalled initiation of the next trial in the series of several hundred which comprised each daily 2 to 3 hour experimental session. Asymptotic levels of performance on this procedure typically required 2 to 3 months of such daily training sessions.

Auditory and visual thresholds were determined by randomly varying (in accordance with the method of constant stimuli) the intensity of the test stimuli from trial to trial and examining detection frequencies (i.e., correct lever releases) at each intensity. For the auditory modality, four intensity levels (10 dB apart) of a 16.0 kHz pure tone were used, with the lowest level set just below the animal's estimated threshold. Interspersed among the test trials were a series of catch trials during which no tone was presented to measure the false alarm (i.e., guessing) rate. For the visual modality, four intensity levels (0.5 log density units apart) of the white light were used with the lowest level again set just below the animal's estimated threshold. Again, catch trials with no light were programmed to occur intermittently. In addition, sessions involving visual threshold determinations were preceded by a 30-min dark adaptation period in the light-proof chamber followed by a 5-min, 50 trial warm up with the highest test intensity of white light.

For both the auditory and visual threshold determinations, each test session was divided into four blocks of approximately 150 trials with each of the 4 intensity levels (plus catch trials) presented randomly approximately 30 times during each block. This provided 4 independent within-session estimates of the sensory thresholds and functions relating reaction time to intensity. Sensory thresholds were determined from percent correct detections at each intensity interpolating to the intensity which produced a detection score halfway between the false alarm rate and 100%. Stable auditory thresholds were based upon determinations from 3 successive test sessions with estimates which varied by no more than 4 dB. Stable visual thresholds were based upon determinations from 3 successive test sessions with estimates which varied by no more than 0.2 log density units. In both cases, such a determination of threshold stability required a false alarm rate below 30% and no systematic change trends in the data. With regard to the response latency measure of reaction time (typically skewed due to the physiological limits on lever release time), the standard measure of central tendency employed for such distribution was the median, with variability reported in terms of the interquartile range.

Following stabilization of the threshold and reaction time measures, preliminary studies were undertaken to explore the validity, reliability, sensitivity, and specificity of these psychophysical methods with respect particularly to the evaluation of pharmacological toxicants. All drugs have been administered intramuscularly at the beginning of each experimental session, followed by a 30-min warm-up before formal threshold determinations were begun. Saline control sessions have been conducted between each drug session and return-to-baseline performances were required during these intervening saline control sessions before further drug administrations were programmed.

Figure 6 illustrates the psychometric function relating percent correct lever releases to intensity in decibels sound pressure level (dB SPL) of a 16 kHz pure tone obtained in the course of a psychophysical experiment with one baboon. As stimulus intensity decreased, percent correct detection also decreased, describing the typical S-shaped function. The absolute threshold, as indicated on Fig. 6, is defined by the stimulus intensity value at which the percent correct detection is halfway between the catch or guessing rate and 100% correct detections. Similar relationships were observed when percent correct detections were determined as a function of white light intensity.

When response latency was measured as a function of stimulus intensity with the baboon performing in a reaction time experiment, the psychophysical functions shown in Fig. 7 were obtained. With both a white light and a 16 kHz tone, response latencies decreased (i.e., reaction time became faster) as stimulus intensity increased. This naturally-occurring relationship between response latency and stimulus intensity required no shaping or training and has been consistently observed in all animals. Additionally, the data plot in Fig. 7 shows that not only do latencies decrease as intensity increases, but the variability in the latency measure also decreases, with the result that trial to trial data replicability was extremely good at the higher stimulus intensities. Finally, Fig. 7 also shows that there was a consistent difference between the response latencies to auditory and visual stimuli. Significantly, at the higher intensities where variability is low, auditory reaction times were consistently found to be approximately 100 msec faster than visual reaction time.

That this basic stimulus intensity–response-latency function may provide a sensitive measure of toxic effects is suggested by the data plot in Fig. 8 which shows the orderly effects of increasing doses of amobarbital sodium (i.e., 3.2, 10.0, 17.0 mg/kg IM) upon reaction time as a function of white light stimulus intensity in the baboon. The latency-intensity functions were recorded 1 to 2 hr after drug administration (i.e., peak action time) and show the systematic relationship between drug dose and response latency at all but the lowest (i.e., threshold) intensity level. Even at the

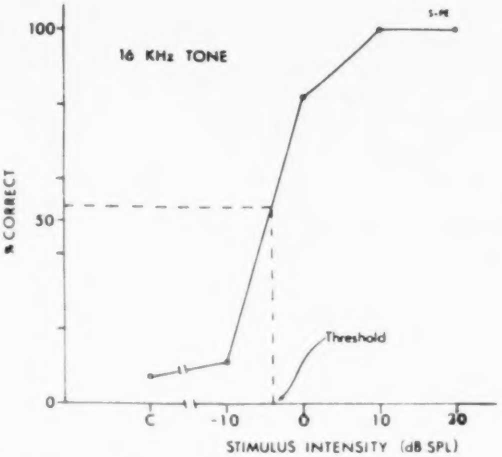

FIG. 6. A psychometric function showing percent correct lever releases to a 16.0 kHz pure tone as a function of stimulus intensity for Baboon S-PE. C = catch trial rate. The indicated threshold is corrected for this catch trial rate (see text).

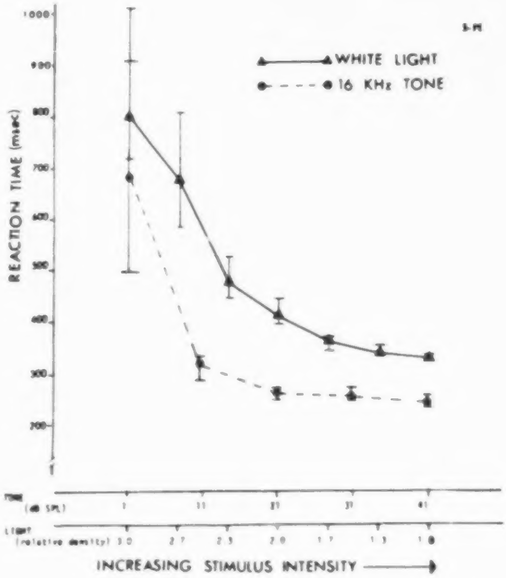

FIG. 7. A latency-intensity function showing median reaction time to either white light (solid lines) or a 16.0 kHz pure tone (dotted lines) as a function of stimulus intensity for Baboon S-PE. Interquartile ranges are bracketed for each point.

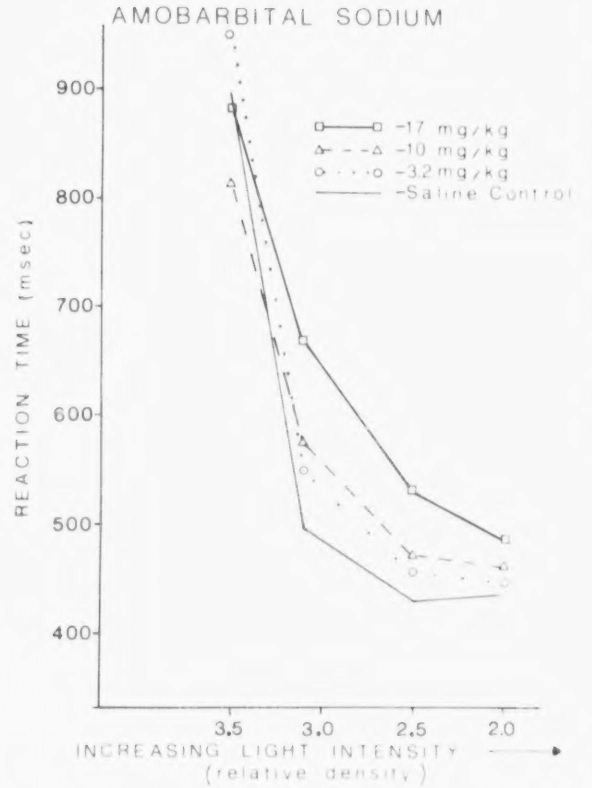

FIG. 8. Latency-intensity functions showing median reaction time to white light as a function of stimulus intensity, with dose level of amobarbital sodium as the parameter. Each curve was obtained at the estimated peak action time of the drug. All data are from one Baboon, S-IK.

highest intensity levels where response variability is minimal (see Fig. 7), the orderly progression of increasing latencies with increasing doses is apparent. The basic psychophysical functions illustrated in Figs. 6 and 7 have been determined and replicated in each of the 4 baboons participating to date in the experiments described below.

Against the background of these basic psychophysical relationships, a series of studies was undertaken to determine the differential sensitivity of the methodology to experimental interventions with predictable effects. Figures 9 and 10, for example, contrast the effects of noise (i.e., 100 dB SPL for 45 min) and pentobarbital (17 mg/kg) upon selective aspects of the animal's psychophysical performance. Exposure to the two independent variables was programmed to occur immediately before the separate experimental sessions scheduled for each intervention, and effects were measured as changes in auditory sensitivity and/or reaction time. Predictably, the data plots in Fig. 9 show an elevation in the threshold sensitivity required for detection of the 16 kHz pure tone during the first 1 to 2 hours following noise exposure. No such auditory threshold shifts were observed following either pentobarbital administration or the non-intervention control procedure. Significantly, experimental sessions which involved visual reaction time performances showed no changes in visual threshold following noise exposure. When the performance measure examined following noise exposure and pentobarbital administration focused upon reaction time (i.e., response latency) however, the effects were quite different. Figure 10 shows the latency increases recorded for each tone intensity during the 1 to 2 hr period following drug administration (i.e., peak action time) as compared to the stable, unchanged reaction times recorded over the same intensity range and time period following noise exposure and the non-intervention control procedure. (The threshold shift following noise exposure made the 0 dB intensity value inaudible in the course of these determinations with the result that no latencies were obtained at this intensity and no point is plotted.)

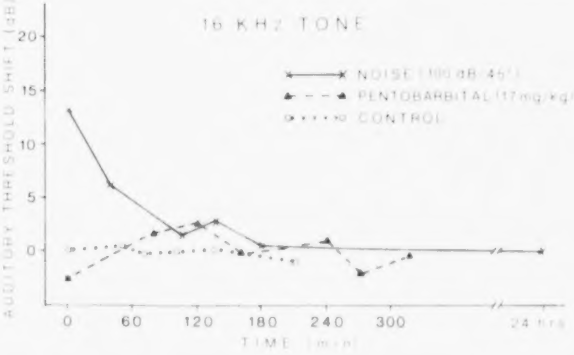

FIG. 9. Changes in the auditory threshold to a 16.0 kHz pure tone as a function of time after 3 different experimental manipulations. (1) Exposure to broadband noise of 100 db for 45 min. (2) I.M. injection of 17.0 mg/kg pentobarbital sodium. (3) No intervention.

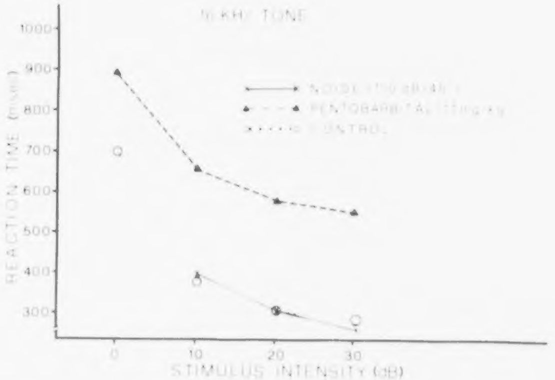

FIG. 10. Latency-intensity functions showing median reaction time to a 16.0 kHz pure tone as a function of stimulus intensity for the same 3 experimental conditions shown in Fig. 9. Each curve was taken from that point in the session showing the peak effect of each of the respective experimental manipulations.

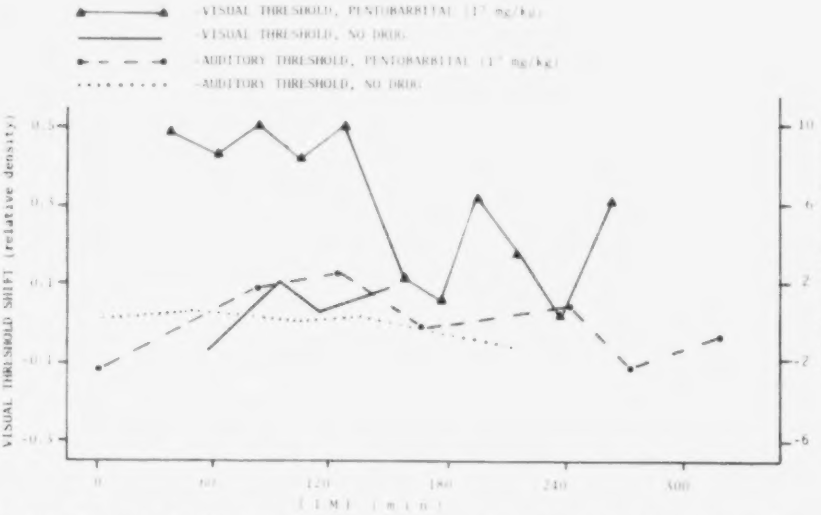

FIG. 11. Changes in the visual threshold to white light and the auditory threshold to a 16.0 kHz pure tone as a function of time after IM administration of 17.0 mg/kg pentobaribtal sodium in Baboon S-PE. Also shown are saline control sessions for both auditory and visual threshold changes. Visual threshold scale is to the left, auditory threshold scale is to the right.

Although no changes in visual sensitivity were observed following noise exposure, pentobarbital administration did significantly elevate visual thresholds. Figure 11 shows the striking elevation in visual threshold during the first 1 to 2 hr of an experimental session following pentobarbital administration, and contrasts these changes with the unaltered auditory thresholds recorded following identical drug exposure in the same animal. Control values recorded during experimental sessions without drug are also plotted for comparison. These preliminary findings support the differential sensitivity of the psychophysical methodology to modality-specific effects and the relative independence and selectivity of the response latency and stimulus threshold measurement components of the procedure.

Figure 12 summarizes the effects of pentobarbital sodium as a function of dose (0, 1.0, 3.2, 10.0, 17.0 mg/kg) upon response latencies and both visual and auditory thresholds in one of two animals showing similarly toxic effects. The indicated reaction time determinations were made with tone and light stimuli at the high end of the intensity distribution where response variability is minimized (see Fig. 7), and during the peak action time of the drug (i.e., 1–2 hr after IM administration). The range of control values obtained during experimental sessions following saline administration is bracketed in each graph by the broken lines labeled saline control range. There were clear effects upon reaction time and visual threshold as a function of dose with both animals, and no change was observed in auditory thresholds over the range of doses studied. For baboon S-PE (Fig. 12), doses of 1.0 and 3.2 mg/kg pentobarbital produced no change in any of the measured functions, though 10.0 and 17.0 mg/kg pentobarbital can be seen to increase response latencies and visual threshold in a dose-dependent manner. No such changes in auditory threshold occurred at any of the indicated doses.

Figure 13 illustrates the effects of diazepam as a function of dose (0, 0.32, 1.0, 3.2, 10.0 mg/kg) upon response latencies and sensory thresholds determined with the same two baboons used in the pentobarbital experiment under the same stimulus conditions and peak drug action time (i.e., 1–2 hr following IM administration) conditions. The range of saline control values is again represented within the broken-line bracketed portion of each graph. With both animals, clear effects upon reaction time and the visual threshold were observed as a function of dose, and auditory thresholds were similarly affected. For the most part, all three effects—increased response latency, visual threshold elevations, and auditory threshold increases—appeared in a dose-dependent manner with progressively greater decrements through the range from 1.0 to 10.0 mg/kg.

The comparative data plots shown in Fig. 14 for baboon S-PE illustrate the typically-observed differences in duration of action between pentobarbital and diazepam for a 10 mg/kg dose of each drug. The previously described effect of both drugs upon reaction time is represented in terms of response latency increases over the course of successive trial blocks (150 trials/block) during a 2–3 hr test session (Day 2) for pentobarbital on the left and diazepam on the right. Within 24 hr, response latencies had recovered to control levels for pentobarbital, as shown by the Day 3 values on the left side of Fig. 14. In contrast, slowed reaction time (i.e., response latency increases) persisted well beyond 48 hr following diazepam administration before recovery of control levels was approximated (Day 5, left side of Fig. 14).

The results of preliminary studies with two additional compounds are summarized in Figs. 15 and 16. Interestingly, observations thus far completed with chlordiazepoxide (Fig. 15) over the same dose range (1.0 to 17.0 mg/kg) used in the pentobarbital (Fig. 12) and diazepam (Fig. 13) experiments, reveal little or no effect of this benzodiazepine compound upon sensory thresholds or reaction time. *d*-Methylamphetamine, on the other hand, appears to produce a selective decrement in visual threshold at the highest dose studied (0.32 mg/kg) with little or no effect on reaction time or auditory threshold (Fig. 16).

The conclusions which can be drawn from these two sets

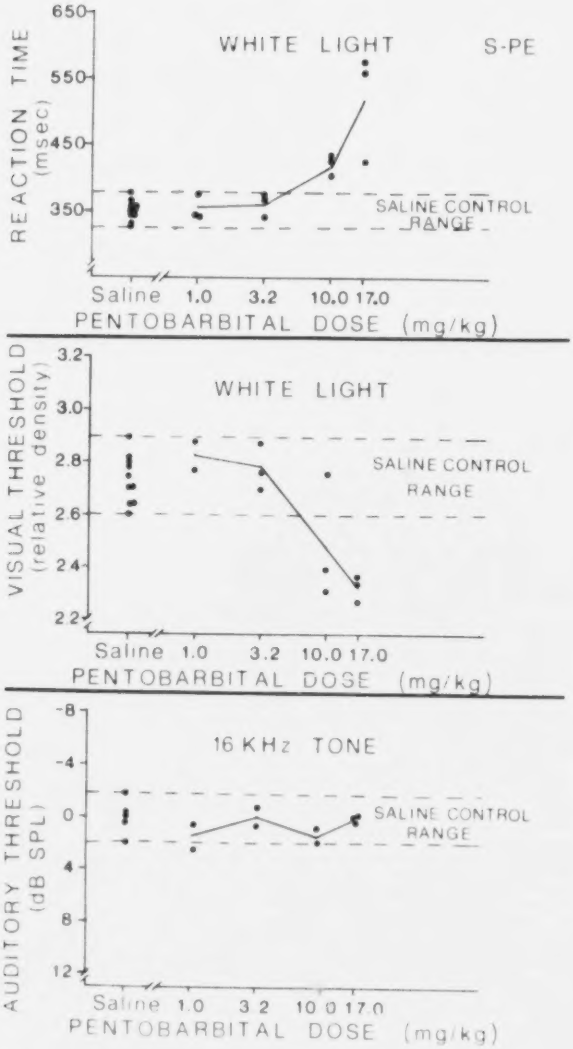

FIG. 12. A three-part figure showing the dose-related effects of pentobarbital sodium on median reaction time to white light (top), visual threshold to white light (middle), and auditory threshold to a 16.0 kHz pure tone (bottom). In each case, the dashed lines bracket the total range in saline control values. All data points were obtained at the peak action time of the drug, and are from the same Baboon, S-PE. Visual reaction time data are medians from the highest stimulus intensity employed.

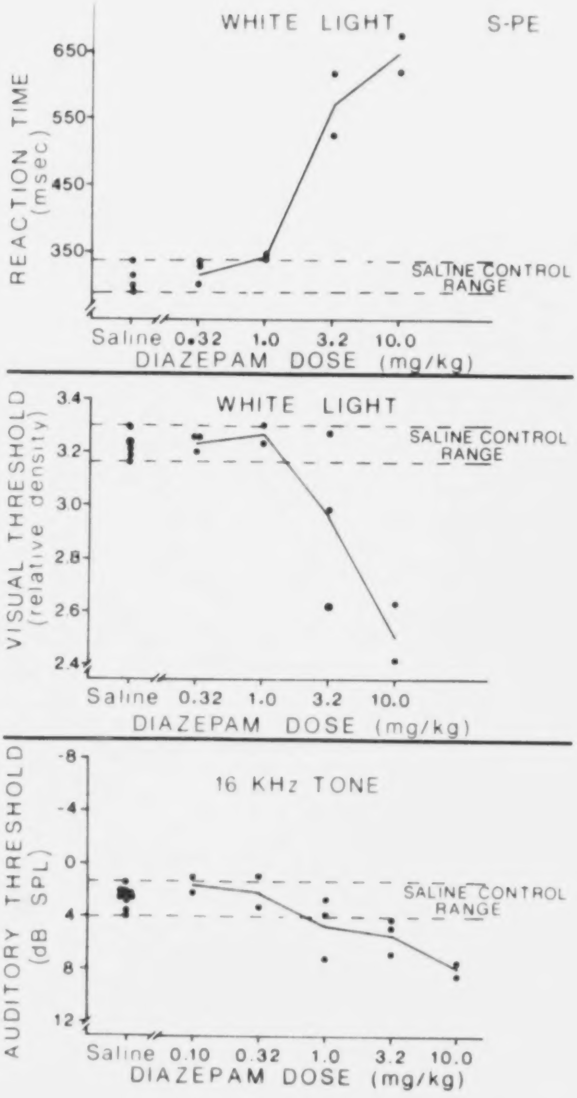

FIG. 13. The dose-related effects of diazepam on median reaction time to white light (top), visual threshold to white light (middle), and auditory threshold to a 16.0 kHz pure tone (bottom) in Baboon S-PE. Further description as in Fig. 12.

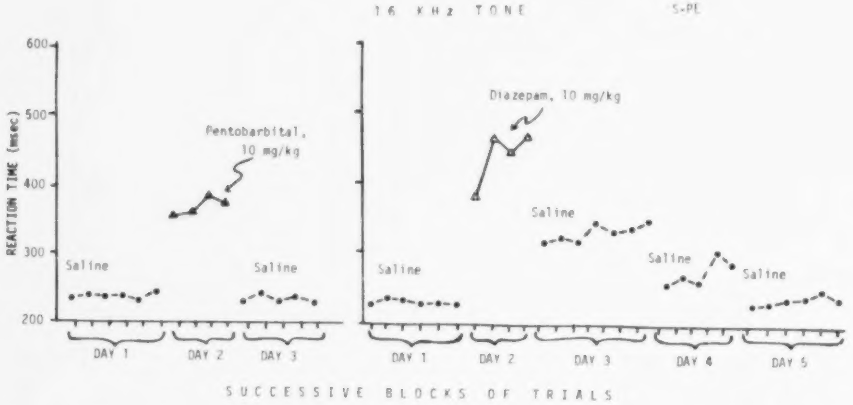

FIG. 14. Changes in median reaction time to a 16.0 kHz pure tone for one Baboon, S-PE, showing the time course of changes in reaction time following IM administration of pentobarbital sodium (10.0 mg/kg) and diazepam (10.0 mg/kg).

of data with respect to the independence of the behavioral processes involved in the risk-taking and psychophysical assessment procedures are, of course, limited. In the first instance, it would appear that overlapping functions may be represented by the response rate and reaction time measures which characterize the effects of toxic substances upon the two assessment methods. The decreases in response rate on the risk-taking procedure and the dose-dependent increases in response latency on the psychophysical procedure following both barbiturate (Figs. 1, 3, 8, 10, 12, 14) and benzodiazepine (Figs. 4, 5, 13, 14) administration are certainly consistent with such a relationship. Close inspection of Fig. 16 also suggests a slight decrease in response latencies at 0.32 mg/kg *d*-methylamphetamine, a finding which accords well with the modest response rate increases observed under similar conditions during risk-taking performance (e.g., Fig. 2). By the same token, it seems unlikely that all of the observed pharmacological toxicant effects can be accounted for by reductionistic appeals to common processes in the two methodological approaches. The selective changes in visual threshold following 0.32 mg/kg *d*-methylamphetamine, (Fig. 16), for example, occurred in the absence of any effects upon yellow or red light response probabilities in the risk-taking procedure under the same drug condition (Fig. 2). And the differential effects of pentobarbital and diazepam upon early and late yellow responding (Fig. 1) and upon low and high shock risk performances (Figs. 3, 4, 5) occurred under conditions where unaffected components of the multi-operant baseline controlled, to some extent at least, for sensory threshold changes. The early and late yellow light stimuli were the same in the experiment shown in Fig. 1 even though performance change occurred only during the late yellow. And the same yellow light stimulus was used for both the low and high shock risk conditions shown in Figs. 3, 4, and 5 even though the performance changes were restricted to the early yellow response rates during low shock risk. Of course, the possibility of complex interaction effects (e.g., between shock probability and visual threshold under drug conditions) can not be ruled out, but it would seem reasonable to conclude on the basis of available data that the risk-taking procedure may provide an approach to assessing the effects of toxic substances upon aspects of a behavioral repertoire which are not necessarily coextensive with sensory-motor processes.

ACKNOWLEDGEMENT

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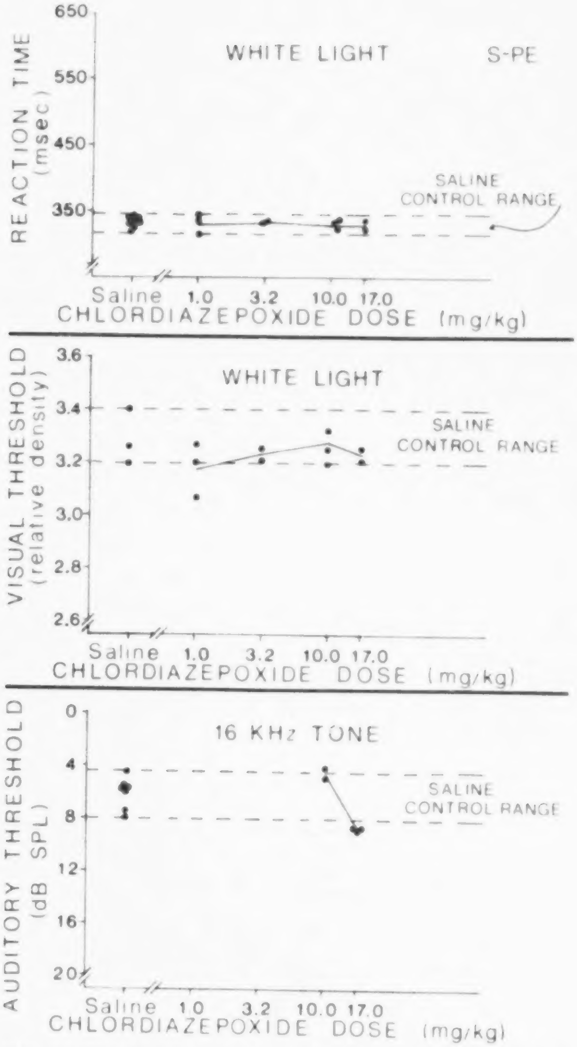

FIG. 15. The dose-related effects of chlordiazepoxide on median reaction time to white light (top), visual threshold to white light (middle), and auditory threshold to a 16.0 kHz pure tone (bottom) in one Baboon, S-PE. Further description as in Fig.12.

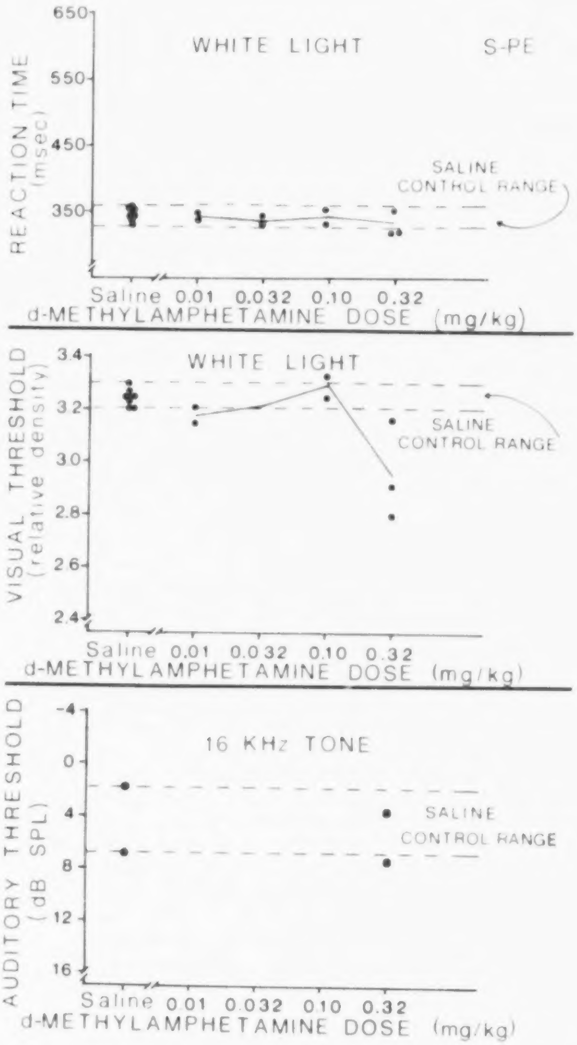

FIG. 16. The dose-related effects of *d*-methylamphetamine on median reaction time to white light (top), visual threshold to white light (middle), and auditory threshold to a 16.0 kHz pure tone (bottom) in Baboon S-PE. Further description as in Fig. 12.

REFERENCES

1. Bradford, L. D., F. Grollman and J. V. Brady. The effects of pentobarbital, diazepam, and *d*-methylamphetamine on conditioned and unconditioned punishment. Presented at *Eastern Psychological Association*, 1979.
2. Evans, H. L. Early methylmercury signs revealed in visual tests. In: *Proceedings of International Congress on Heavy Metals in the Environment*, Vol. 3, edited by T. C. Hutchinson. Toronto, Canada: Institute for Environmental Studies, 1977.
3. Hienz, R. D., F. Grollman and J. V. Brady. Psychophysical assessment of drug effects on sensory and motor processes in the baboon. Presented at *Eastern Psychological Association*, 1979.
4. Laties, V. G., P. B. Dews, D. E. McMillan and S. E. Norton. Behavioral toxicity tests. In: *Principles and Procedures for Evaluating the Toxicity of Household Substances*. Washington, D.C.: National Academy of Science, 1977, p. 111.
5. Stebbins, W. C. and S. Coombs. Behavioral assessment of ototoxicity in nonhuman primates. In: *Behavioral Toxicology*, edited by B. Weiss and V. G. Laties. New York: Plenum Publ. Corp., 1975, p. 401.
6. Weiss, B. The behavioral toxicology of metals. *Fedn Proc.* **37:** 22–27, 1978.
7. Weiss, B. and V. G. Laties. *Behavioral Toxicology*. New York: Plenum Publ. Corp., 1975.
8. Wood, R. W., A. B. Weiss and B. Weiss. Hand tremor induced by industrial exposure to inorganic mercury. *Archs Envir. Hlth.* **26:** 249–252, 1973.

Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function
Neurobehavioral Toxicology, Vol. 1, Suppl. 1, pp. 85–92. ANKHO International Inc., 1979.

Operant Conditioning of Infant Monkeys *(Macaca fascicularis)* for Toxicity Testing

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RICE, D. C. *Operant conditioning of infant monkeys* (Macaca fascicularis) *for toxicity testing.* NEUROBEHAV. TOXICOL. **1:** Suppl. 1. 85–92, 1979.—A technique has been developed that allows infant monkeys to perform on an operant schedule as soon as they are able to self-feed. Behavior is shaped in small increments through a series of operants: sensory and motor systems as well as performance on schedules using intermittent reinforcement may be tested as early as 3–4 weeks of age. This is accomplished by exposing the infant to the operant situation almost continuously, and allowing the infant to feed only by operantly responding. Infants exposed to lead post-natally differed from controls in pattern of fixed ratio responding, "activity" as measured by pattern of responding over the course of the session, and on a two-choice form discrimination reversal learning set paradigm. This technique allows rapid accumulation of large amounts of data without experimenter intervention.

Operant conditioning   Infant monkey   Visual discrimination   Discrimination reversal   Fixed ratio   Activity   Lead   Neonatal exposure

THE developmental study of perception and learning in newborn animals is of interest to investigators in many areas of research, including behavioral toxicology. Even though in many cases the primate offers the best model for understanding the functioning of the human nervous system, there have been only a few studies using operant responding to study development of infant primates. Reasons for the lack of information on young primates include the poor motor coordination in the very young primate as well as the necessity of monitoring the infant in its home environment under unrestricted conditions.

Recently an operant technique that allows collection of large amounts of data on infant rhesus or pigtail macaques performing in their home cage under ad lib motivation conditions has been used to study the development of the visual system [1,4]. This paper describes modifications to this basic technique that allow a relatively large number of infant monkeys to be studied up until one year of age or longer with a minimum amount of electronic and mechanical maintenance.

METHOD

Apparatus

Monkeys were housed from Day 1 of life in stainless steel cages (38.1 × 48.3 × 48.3 cm), with a wire mesh floor and pan underneath to collect urine and feces (Fig. 1). Pairs of cages were supported by an angle iron frame with wheels. Each cage opened from the top. The center of the front of the cage was cut away, surrounded by slides so that "fronts" of different types could be attached easily and adjusted to any height. Fronts were of two types: 0.63 cm stainless steel bars spaced 3.8 cm apart, and a clear Plexiglas sheet containing a "mask" and touch-bar attachment (Fig. 2). The mask consisted of a hemisphere with eye and mouth holes, with arm holes beneath, made from mouth guard material. The first mold was made by inserting a rubber ball halfway into Jeltrate caulk: a positive was then made from dental cement to be used as the template for the mouth guard material, which was melted over the cast in an oven.

The behavioral apparatus was mounted on a frame made from aluminum rods held together by Flexiframe clamps (Fisher Scientific Co., Pittsburgh, PA), so that all levers, feeders, etc., were independently adjustable in three dimensions. This frame was also on wheels and was attached to the cages by a rod fitting into a holder at either end of a pair of cages. The frame containing the electrical equipment therefore could be disconnected easily from the housing units. With the exception of the Plexiglas fronts, the cages were washed by the automatic cage-washing equipment used by the primate facility.

The feeding apparatus for infants under 30 days of age consisted of a 110 V AC pull-type solenoid to which 0.32 cm stiff Teflon tubing with a rubber nipple at one end could be attached with clamps (Fig. 3). This tubing rested inside a Plexiglas tube which fit up against the mask, thus protecting the Teflon tubing from being grabbed and pulled into the mouth hole by the monkey. The Teflon tubing was attached by flexible tubing to a reservoir which sat below the level of the nipple. Operation of the solenoid moved the nipple into the mouth hole, thus allowing the monkey to suck. Backflow into the reservoir was prevented by a one-way glass valve in the line.

Infants over 30 days of age were reinforced with a small

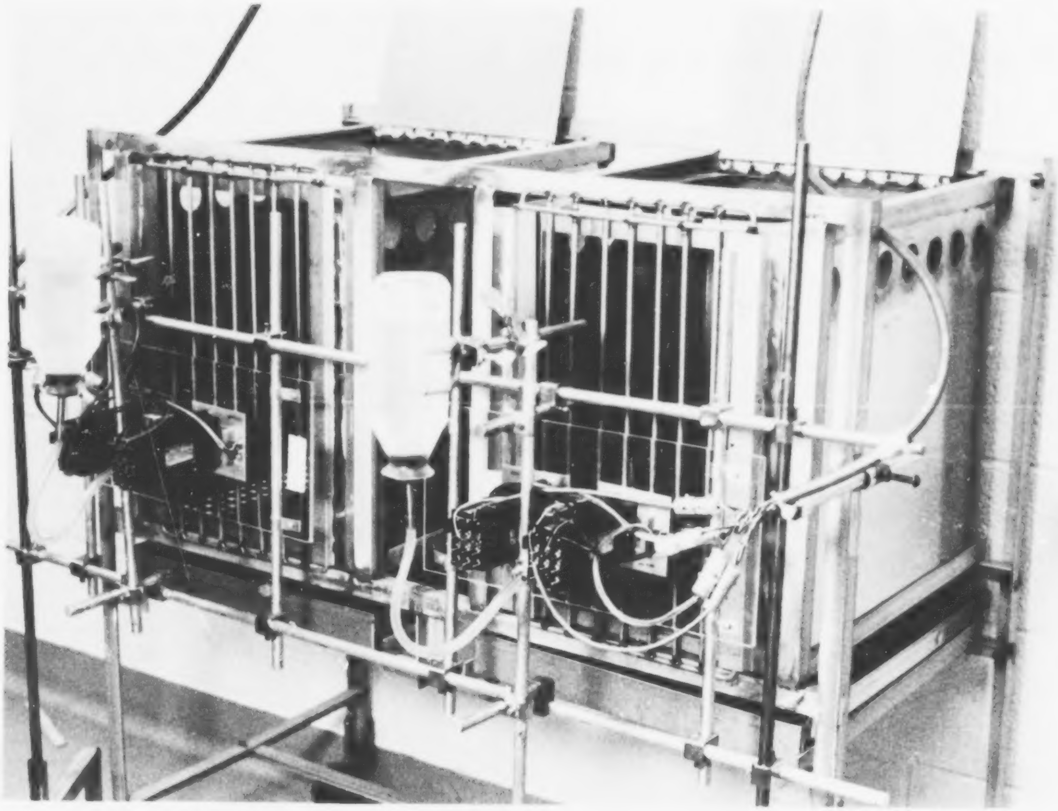

FIG. 1. Pair of stainless steel units for operant conditioning of infant monkeys, with steel bar fronts and feeders with steel drinking tubes. This configuration is for older infants responding on push-butto

amount of formula delivered by a 110 V AC Skinner (Skinner Precision Industries, Inc., New Britain, CT) two-way solenoid valve connected to a steel drinking tube which protruded about 2.5 cm into the cage.

Control of experimental procedures and data collection were performed using the state notation language SKED [3] operating on a Nova 3 minicomputer (Data General Corporation, Southboro, MA). Data were collected by recording every interevent time to the nearest 10 msec; thus, a session could be precisely reconstructed from the raw data.

Experimental Procedure

The floor of the cage was covered with paper diapers for young infants, and all infants had a surrogate attached to the side of the cage and a paper diaper free in the cage. A heating pad was used for the first few days of life.

Infants were housed in the cages 24 hours a day with access to infant formula via a rubber nipple until they learned to self-feed. Nursery personnel held them to the nipple on a fixed schedule until they learned to find it themselves. After they had been self-feeding for three days, they were exposed to the following sequence of schedules:

(1) The infant interrupted a photocell beam by placing its face in the mask in order to gain access to the nipple for 10 sec. The beam had to remain interrupted for 0.2 sec (2 sessions), 0.5 sec (2 sessions), or 1 sec (2 sessions). If the beam were broken when the 10 sec feed time ended, the infant could simply keep it broken for the specified time in order to gain further access to the nipple.

(2) The infant touched a "touch bar" (contact sensor) directly outside the arm holes while the mask photocell was interrupted. The infant could use either hand, and left and right touches were recorded separately. Initially contact with the touch bar was made accidentally when the infant grabbed the arm holes for support while feeding. The touch bar was moved further away from the Plexiglas front on successive days so that the monkey had to reach progressively further to respond. Infants that would be required to use both hands had one arm hole blocked every session in an alternating fashion once they had used their preferred hand for 2 sessions. The touch bar was moved 1.26 cm every other night; they were required to respond with their preferred and then non-preferred hand at each bar placement. Infants that would respond with one hand (such as on a fixed ratio schedule) were allowed to use only their preferred hand.

(3) At 30-40 days of age, the rubber nipple was replaced

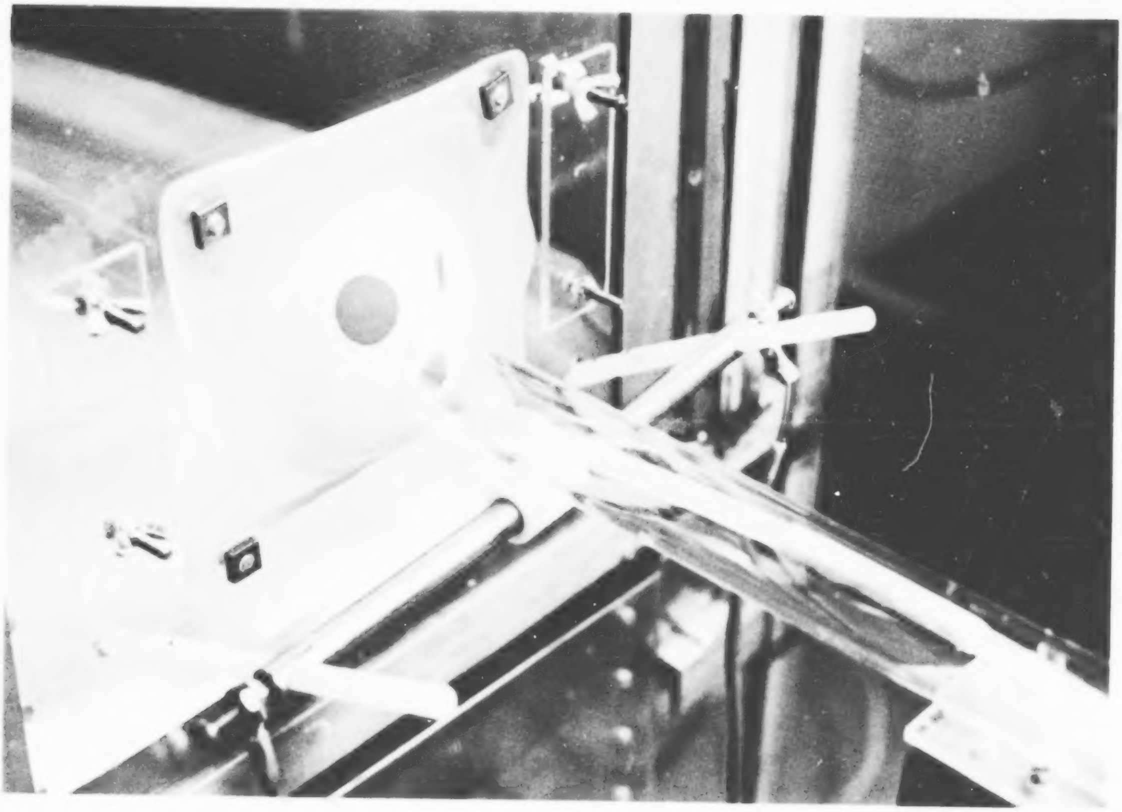

FIG. 2. Close-up of Plexiglas front with mask, touch bar attachment, and rubber nipple feeder. The arm hole is on the side not visible.

with a drinking tube, and the photocell was no longer used; 0.2 ml of formula was delivered at each reinforcement.

(4) When the infant worked with its preferred hand or either hand with the bar 5 cm from the front of the cage, a push button with a colored light behind it was substituted for the bar on the side of the preferred hand. Infants typically pushed it spontaneously with no shaping necessary. Infants performed on the push button for two sessions using the preferred hand or two sessions with each hand.

(5) Once the infant responded on the push button, it was put on one of two schedules. One of these was a non-spatial discrimination, in which the final form discrimination was shaped by a series of easier tasks. Infants were first introduced to a red-dark discrimination. When they performed at 85% or above correct for five sessions, a green light was introduced as the negative stimulus. When the same criterion was met (85% correct or better for five sessions), a cross was superimposed on the positive stimulus and a triangle on the negative. When criterion was met, the colors were removed. A series of up to 10 reversals were then performed, the criterion for reversal again being 85% correct or better for five sessions.

The other schedule was a fixed ratio (FR) 1 with the infant using its preferred hand, followed by FR10, 20, 30 and 40. Each FR value was tested for 14 sessions. Infants were moved from FR1 to FR10 using intermediate FR values over the course of three sessions.

The eating pattern (or response pattern) of each infant throughout the course of the session was also determined.

Until the infants were 45 days old, sessions were 21 hours long, from 3 p.m. to 12 noon. Sessions were then 16 hours long, from 3 p.m. to 7 a.m. All infants received all their formula during the session; there were no supplemental feedings. Infants also received fresh fruit and primate diet as per standard nursery procedure.

When infants were not performing on an operant schedule, they were housed in standard nursery clear polycarbonate housing units. Peer socialization began at 20 days of age; infants were placed in pairs in these housing units for one hour each day. They were gradually introduced in small groups to the large exercise cages, and allowed to remain for increasing periods of time. At 45 days of age, when they were in the operant cages for 16 hours overnight only, they were housed in the large exercise cages with monkeys around their own age for approximately five hours per day two or three times a week.

RESULTS

Once the infant was self-feeding, there was a minimum of

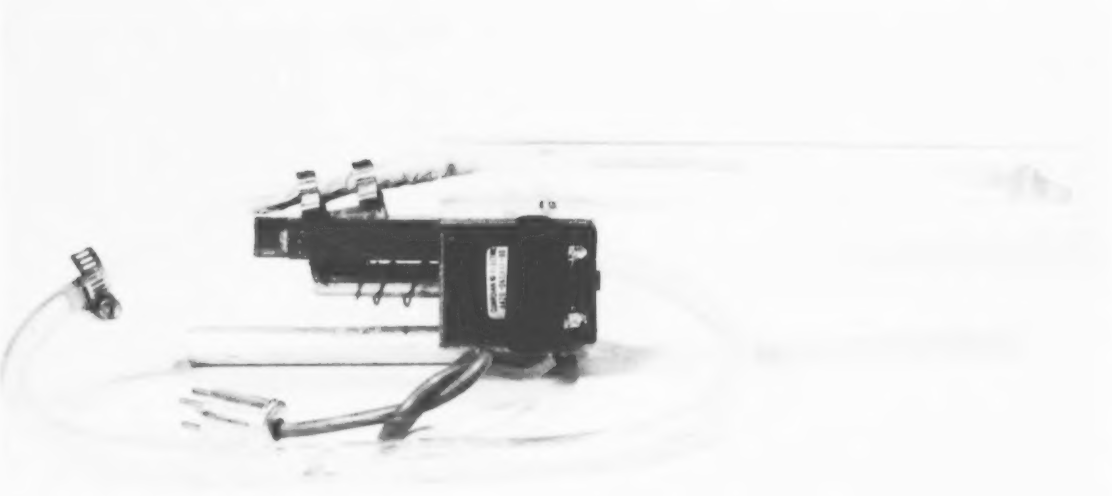

FIG. 3. Rubber nipple feeder with pull-type solenoid. Solenoid activation pulls nipple forward; spring returns it to original position. Note one-way glass valve to prevent backflow of formula.

TABLE 1

NUMBER OF SESSIONS (DAYS) FOR EACH INFANT TO PROGRESS THROUGH SERIES OF OPERANTS REQUIRED TO LEARN TO PRESS THE BUTTON. THE NUMBER IN THE "SELF-FEED" ROW REFERS TO THE AGE OF THE INFANT WHEN IT WAS ABLE TO FIND THE NIPPLE BY ITSELF. THE "MASK" PROGRAMS REQUIRED THE INFANT TO INTERRUPT A PHOTOCELL BEAM ACROSS THE FACE MASK IN ORDER TO GAIN ACCESS TO THE NIPPLE; THE "TOUCH" PROGRAMS REQUIRED THE MONKEY TO TOUCH A CONTACT SENSOR WHICH WAS MOVED PROGRESSIVELY FARTHER FROM THE CAGE FRONT. THE "PUSH-BUTTON" PROGRAMS REQUIRED THE INFANT TO PRESS A BUTTON WITH A RED LIGHT BEHIND IT, FOR TWO SESSIONS WITH EACH HAND. THE NUMBER IN THE "MINIMUM REQUIRED" COLUMN REFERS TO THE MINIMUM NUMBER OF SESSIONS POSSIBLE TO PROGRESS THROUGH THE SERIES.

Schedule	Minimum Required	No. 86	No. 88	No. 89
Self-Feed		Day 3	Day 7	Day 5
Mask	6	6	7	6
Touch Bar	16	8 (preferred) 17 (both)	19	16
Push Button	4	4	4	4

26 (both hands) or 16 (one hand) sessions required until the push button response was established. Of the first three monkeys exposed to the procedure requiring responding with both hands, one completed the series in the minimum number of sessions and another required four extra sessions (Table 1). These results are typical of infants exposed to this regimen (now numbering 26 infants). The third infant was allowed to use only its preferred hand until it acquired the push button response; an unsuccessful attempt was then made to transfer this response to the non-preferred hand. The touch bar was then introduced on the non-preferred side, and the sequence for two hands was initiated.

This method of shaping the behavior of the infants in small increments insured that the formula consumption of the infants did not suffer. This is clear from a comparison of the three pilot infants with the three non-operant infants closest in age (Fig. 4). In fact, infants required to make an operant response in order to receive food actually consumed more formula than those fed ad lib. Ad lib control monkeys were offered a total of 240 ml of formula per day, while the amount of formula consumed by the infants on the operant study was not limited. This could artificially lower the consumption for the ad lib control monkeys. Maximum formula consumption occurred in 4% of feedings for one monkey, 10% for another, and 18% for the third. This was not a factor, however, until at least 90 days of age.

After infants learned the push-button response with either hand, they responded readily on the first session of the light-dark discrimination. Infants learned each discrimination in an orderly fashion with progressive improvement in performance across sessions, as demonstrated by the three pilot infants (Fig. 5). These untreated infants also tended to have fewer errors on the first session of successive reversals.

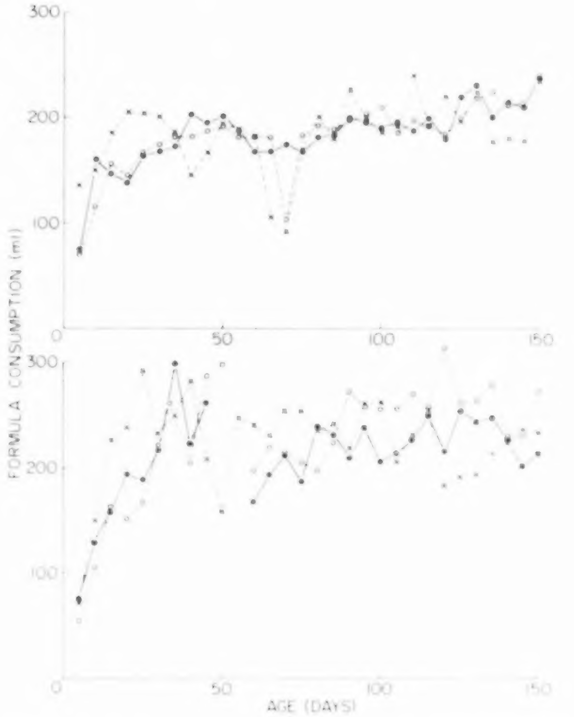

FIG. 4. Formula consumption for three nursery control (top) and operant (bottom) infants. Each point represents the average of 5 days. The break in the graphs represents time when monkeys were not on an operant schedule to allow for computer hardware changes. The drop in consumption for two of the nursery control monkeys around 60 days of age was due to changing from rubber nipples to steel drinking tubes.

Towards the end of the experiment, performance on non-reversal sessions was typically between 90–95% correct.

Monkeys exposed to low levels of lead from birth showed a small deficit in a series of 9 reversals of the form discrimination. They tended to have a lower percentage of correct responses on the first session of a reversal, as well as more incorrect responses after the first incorrect response. This was especially true for the later reversals. For example, the lead monkeys had a significantly lower percentage of correct responses and a higher number of "perseverative" errors for the second block of 50 trials for the last three reversals than do controls ($p < 0.05$, analysis of variance, split plot design). Additionally, the interaction term was significant for the "perseverative" errors ($p < 0.001$) (Fig. 6).

Before working on the form discrimination, these same infants performed on fixed ratio (FR) schedule, with FR values from 1 to 40. The lead-treated monkeys tended to have shorter interresponse times (IRT's), which was reflected in the FR run times (time it took to complete the FR once the monkey started to respond—Figs. 7 and 8). This was especially striking for the two treated monkeys with the highest blood lead levels.

Since the data were collected as interevent times, the "activity" of the infants could be monitored across the 16-hour session. Figure 9 represents the change in the pattern of responding of a normal infant between 15 and 200 days of age. The temporal distribution of responding throughout the course of the session reveals that the infants feed often during the night, without long stretches of no responding. This is especially true until approximately 120 days of age; rarely does more than an hour pass without a feeding bout. As the infant gets older, more formula is consumed per feeding (as measured by number of responses) and the interval between feedings increases. By six months of age, infants often respond in four or five long bouts during a 16 hour session.

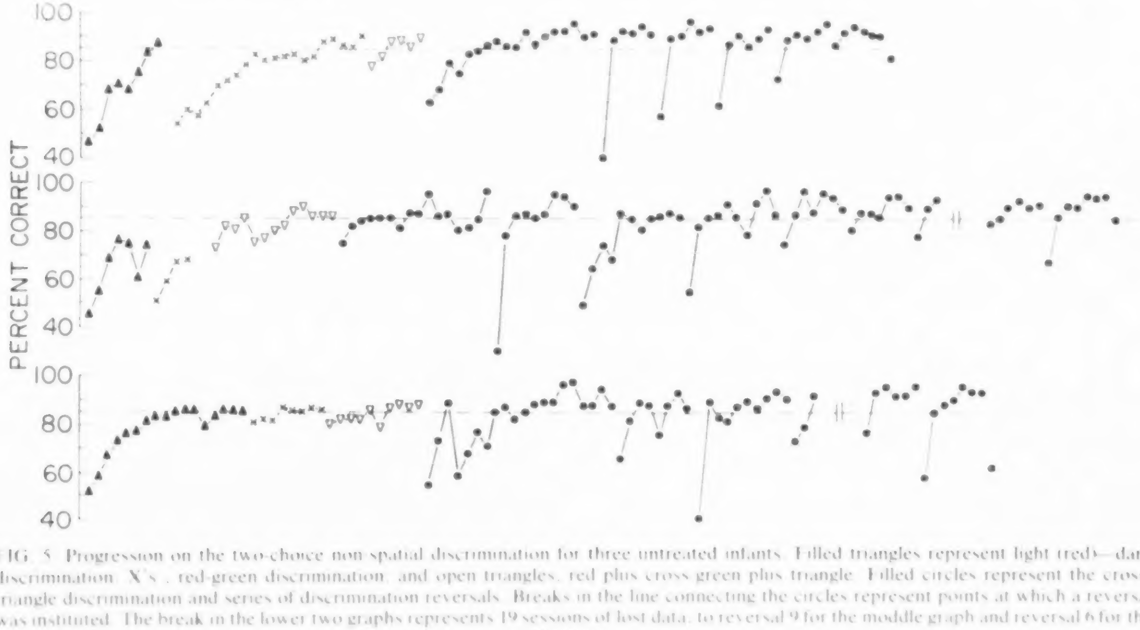

FIG. 5. Progression on the two-choice non-spatial discrimination for three untreated infants. Filled triangles represent light (red)—dark discrimination. X's, red-green discrimination, and open triangles, red plus cross-green plus triangle. Filled circles represent the cross-triangle discrimination and series of discrimination reversals. Breaks in the line connecting the circles represent points at which a reversal was instituted. The break in the lower two graphs represents 19 sessions of lost data, to reversal 9 for the moddle graph and reversal 6 for the bottom.

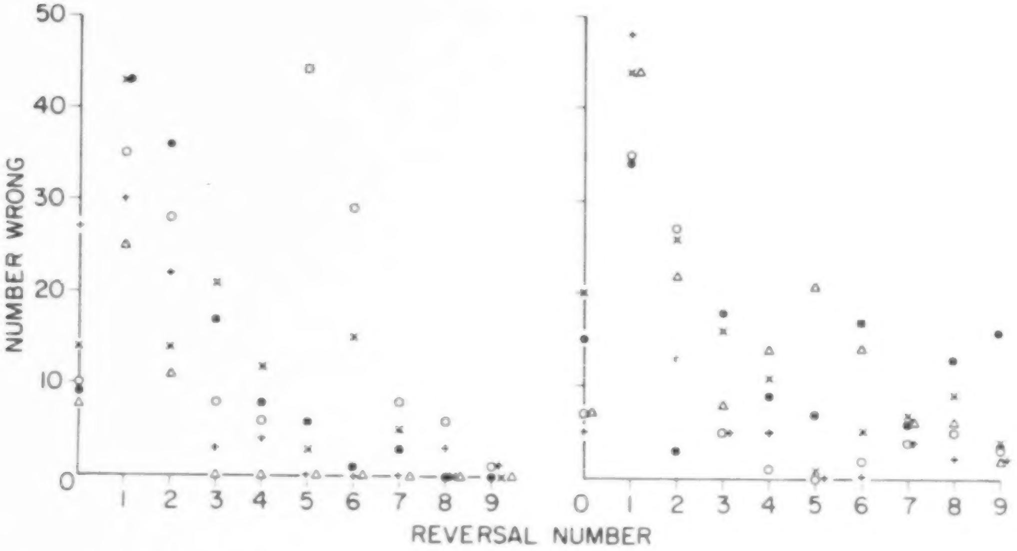

FIG. 6. Number of errors after the first for the second block of 50 trials in the first session of a reversal. Left, control monkeys; right, treated. Each symbol type represents a different animal.

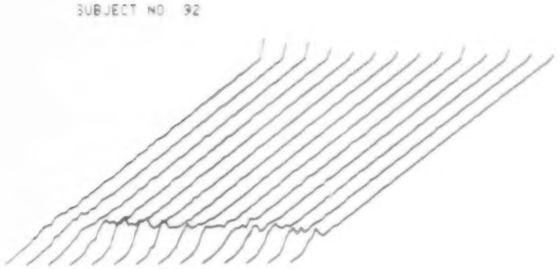

FIG. 7. Histograms of FR run times for the 14 sessions of FR10 for a control monkey. Sessions are ordered from left to right; run times from bottom to top. Z axis represents 35 sec in 0.5 sec bins.

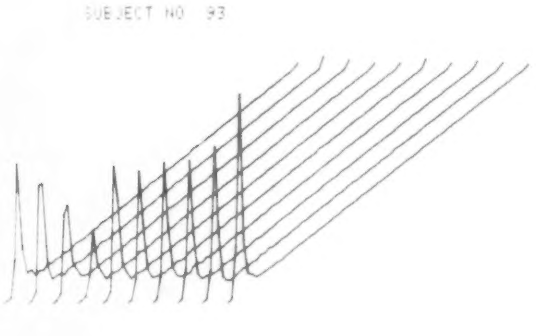

FIG. 8. Histograms of FR run times for the FR10 sessions for a lead-treated monkey. Axes as in Fig. 7.

This developmental pattern is quite consistent among untreated infants examined thus far.

The activity pattern over the course of the sessions in which the infants were responding on an FR40 (approximately 120 days of age) for a control and lead-treated monkey are in Figs. 10 and 11. The control monkey exhibits the typical pattern of four to six eating bouts per session, while the lead-treated monkey feeds more often and drinks less per bout. Nor was this likely a function of delayed development, as this pattern of responding persisted until these monkeys were taken off operant testing at 270 days of age.

DISCUSSION

This paper describes a method for operantly conditioning infant primates starting as early as the first week of life. The procedure has several advantages:

(1) The cage design allows the electrical equipment to be detached from the housing unit, and the cage itself is easily cleaned by automatic equipment using high temperatures. Maintenance of a clean environment is also facilitated by all cabling being away from the floor or walls.

(2) The use of the touch bar allows shaping of the pushbutton response without experimenter intervention. This not only saves time, but also insures that each infant is shaped by the same procedure, thus eliminating uncontrolled variables introduced by experimenter-subject interaction. The small increments of the shaping procedure also insure that the infant will progress through the series without undue stress or weight loss.

(3) The fact that the infants receive all formula in the experimental chamber over a long (at least 16 hr) session obviates the need for restriction of food or water intake. This is especially important in the young animal, since early nutritional deprivation may have detrimental effects on intelligence in humans [2,5] and primates [6].

Sessions were run during the night because experience in

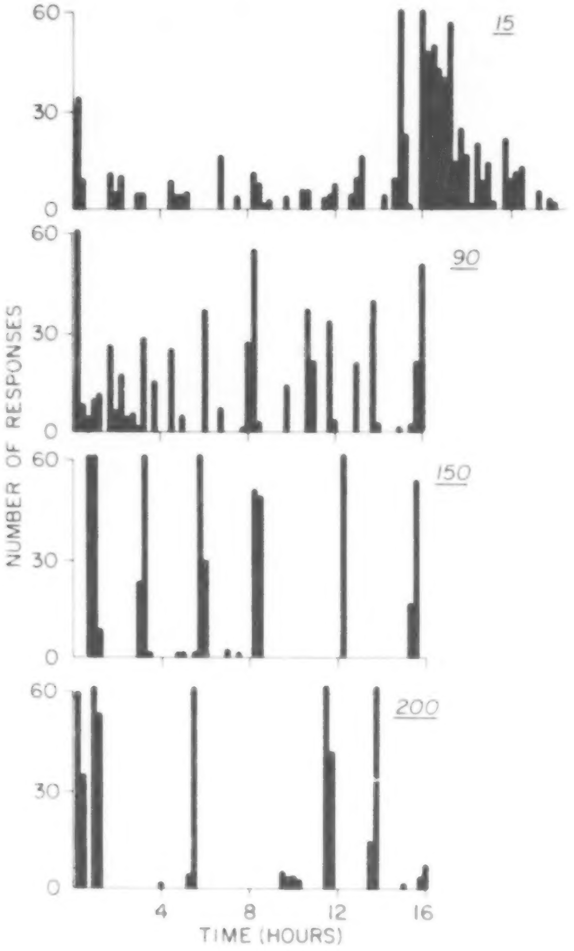

FIG. 9. Response distribution pattern over the course of a session for an untreated infant 15, 90, 150 and 200 days of age. Points on the ordinate represent successive 15 minute segments of the session; the abscissa is number of responses per 15 min segment. All responses exceeding 60 in a 15 min segment are excluded.

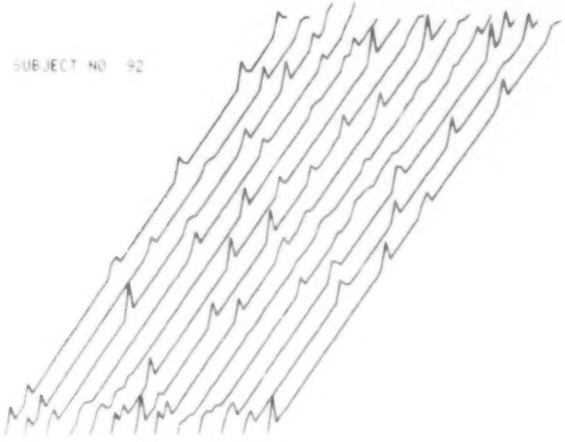

FIG. 10. Histograms of activity across the FR40 sessions for a control monkey. Z-axis represents time into the session in 15 min increments. Y-axis is number of reinforcements.

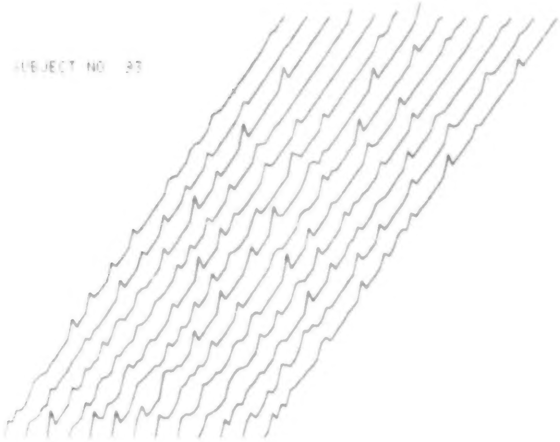

FIG. 11. Histograms of activity across the FR40 sessions for a lead-treated monkey. Axes as in Fig. 10.

nursery rearing infants has demonstrated that this is the period in which they consume the major portion of their daily formula intake. This procedure has the added advantage of freeing the infants during the day for peer socialization, observation, other sorts of toxicity testing, etc. Cleaning and maintenance of equipment can also be done without interfering with the operant sessions.

This study demonstrates that infant macaques may be operantly conditioned as soon as they can self-feed. The method described allows the study of perceptual and learning functions very early in the neonatal primate's life, while the fact that the infant performs in its home cage under ad lib motivational conditions eliminates stress due to food deprivation, strange surroundings, etc. This is especially relevant for perinatal exposure to pharmacologic or toxicologic agents, as these sorts of stress may interact with the toxic effects of the agent.

REFERENCES

1. Booth, R., D. Teller and G. Sackett. Trichromacy in normally reared and light deprived infants (*Macaca nemestrina*). *Vis. Res.* **15:** 1187–1191, 1975.

2. Cravioto, J. and B. Robles. Evolution of adaptive and motor behavior during rehabilitation from kwashiorkor. *Am. J. Orthopsychiat.* **35:** 449, 1965.

3. Gilbert, S. and D. Rice. Nova SKED: A behavioral notation language for Data General minicomputers. *Behav. Res. Metho. Instrum.* **10:** 705–709, 1978.
4. Sackett, G., R. Tripp, C. Milbrath and J. Gluck. A method for studying visually guided perception and learning in newborn macaques. *Behav. Res. Meth. Instrum.* **3:** 233–236, 1971.
5. Stoch, M. and P. Smythe. Does undernutriton during infancy inhibit brain growth and subsequent intellectual development? *Arch. Dis. Childhood* **38:** 546, 1963.
6. Zimmerman, R. and D. Geist. Attention deficiencies in malnourished monkeys. In: *Symposia of the Swedish Nutritional Foundation, XII.*, edited by J. Cravioto, L. Hambraeus and B. Vahlquist. Uppsula: Almqvist and Wiksell, 1974, pp. 115–126.

Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function
Neurobehavioral Toxicology, Vol. 1, Suppl. 1, pp. 93–103. ANKHO International Inc., 1979.

Effects of Pre- and Post-Natal Lead on Affective Behavior and Learning in the Rat[1,2]

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FLYNN, J. C., E. R. FLYNN AND J. H. PATTON. *Effects of pre- and post-natal lead on affective behavior and learning in the rat.* NEUROBEHAV. TOXICOL. **1:** Suppl. 1, 93–103, 1979.—Literature relevant to the relationship between early ingestion of inorganic lead and subsequent hyperactivity in rodents is discussed. Original research in the area is presented. Rats so exposed were not hyperactive in any of the situations investigated or under any of the dosage regimens employed. They did show hypoactivity in the open field when dosed over a prolonged period. Using a new behavior measure, lead-treated rats were found to be less active than controls in the passive avoidance situation. The possible utility of this new measure for behavioral and developmental toxicology is discussed. It is concluded that the available evidence does not support the contention that a meaningful relationship exists between early lead ingestion and hyperactive behavior. It is suggested that future research may more profitably be directed to assessing the effects of lead ingestion on behavior in stressful or fear provoking situations.

Lead　Hyperactivity　Affective behavior　Passive avoidance learning　Social interaction
Behavioral toxicology

THE demonstration that a relatively low body burden of inorganic lead may be of etiological significance in childhood hyperactivity [3,4] is a matter of considerable interest to toxicologists. This is especially true in view of a number of recent reports [22,34] indicating that hyperactivity can be induced in rats and mice as a result of the post-natal administration of inorganic lead, in amounts less than those which lead to obvious symptoms of lead intoxication. Whether such reported hyperactivity is indeed analogous to that demonstrated by the hyperkinetic child or the child diagnosed as exhibiting minimal brain dysfunction (MBD) is a matter of considerable scientific and clinical import. If true, an animal model (or at least a first approximation thereof) of a perplexing clinical entity will have been found. Such a possibility has been suggested [35,42]. The research reported in the present paper suggests that the presumed relationship between early lead ingestion and subsequent hyperactivity in rats is not a simple one, and is a weak relationship, at best. When proper controls are imposed upon the experiment, either no relationship between early lead ingestion and later hyperactivity is found, or the relationship is so small as to be of little predictive utility. At times, an inverse relationship is found, in which lead exposed animals are less active than control animals.

Much of the work on lead and hyperactivity comes from two laboratories. This work will be reviewed in some detail. It has served as a prototype for much of the later work in the field, and it has provided a research paradigm that is of considerable interest to developmental and behavioral toxicology. Specifically, this work involves the exposure of developing rats or mice to lead via the maternal milk supply. Lactating dams are provided with inorganic lead salts in their diet or drinking water. Nursing neonates are thereby exposed to relatively low concentrations of lead by virtue of suckling these dams. Throughout our review of these studies we will direct attention to two major methodological points. The first of these is the necessity, in this research paradigm, of pair-watering or pair-feeding the control animals, as a minimal control for the effects of malnutrition or dehydration. When lactating dams are presented with adulterated food or water they eat or drink less than control animals [30]. Their body weights decline, as does the body weight of their offspring [30]. Malnutrition has been shown to produce hyperactivity in rats [39,40].

The second methodological point stressed is the necessity of removing from the dependent variable, by either experimental or statistical means, apparent treatment effects which are in reality effects that result from running intact groups.

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[1] Supported in part by grants to John C. Flynn by the Baylor University Research Committee.
[2] The authors are grateful to Sterling Winthrop Research Institute for supplying the MMTA.

That is, it is necessary to control for pre-existing litter differences in this kind of developmental study. The necessity for this type of control is well known in behavioral research and has been forcefully stated [6]. In some of the studies reviewed, reporting of experimental procedures and statistical analysis is fragmentary. It is, therefore, sometimes difficult to be certain that controls for litter effects were employed.

In one series of experiments the focus has been on the role of lead in producing hyperactivity in developing mice. In the first of these studies [34] suckling mice were raised by dams whose drinking water contained lead acetate, $Pb(Ac)_2$, in concentrations of 2 mg/ml, 5 mg/ml, or 10 mg/ml. Lead treated offspring were reported to be three times more active than the offspring of dams who drank a sodium acetate (NaAc) solution. Control dams in this study were not pair-watered. Development was retarded in the lead-treated animals, and some of them showed ataxia and disturbed gait. It does not appear that litter effects were controlled in this study.

In a similar study [35] where experimental dams received $Pb(Ac)_2$ while controls received NaAc, ataxia and disordered gait are again reported in the lead-treated offspring. In fact, while an increase in activity is reported for the lead-treated mice, the authors state that activity was measured in animals chosen for an absence of motor symptoms. Such nonrandom selection of subjects provides additional opportunity of observing spurious treatment effects. Lead animals are reported to respond "paradoxically" to d-amphetamine and phenobarbital in a manner consistent with the presumed response of hyperactive children to these drugs [17,43]. No growth data are presented for the developing mice. Pair-watering was not utilized, and litter effects apparently were uncontrolled.

In a subsequent study [36] these authors report on several neurochemical studies of the lead exposed mouse. Effects on the high affinity uptake of certain neurochemicals are reported. Also, lead-treated animals are reported to differ from controls in their response to a variety of pharmacological agents. We will return to biochemical and pharmacological considerations below.

In another series of studies the focus has been on the relationship between lead and hyperactivity in rats. In the first report [32] lactating rat dams were fed powdered laboratory chow containing 4.0% lead carbonate, $Pb(CO_3)_2$. In this study, control dams were pair-fed. The activity of entire groups of offspring was measured, rather than individual activity. The offspring of lead mothers were said to show a 40% to 90% increase in activity when compared to controls. While pair-feeding was used in this experiment, it is probable that litter differences were not controlled. Lead animals also were reported to show a 20% decrease in brain dopamine (DA) relative to controls, but were not different from controls in brain norepinephrine (NE). In a similar study [22] these authors report on the activity of the offspring of dams consuming powdered chow containing 5.0% $Pb(Ac)_2$. Group measures of activity were again used, and lead animals were found to be more active than controls. Pair-feeding was again employed. The necessity of so doing was demonstrated in this study by comparative growth curves. Unfortunately, there is no evidence of any attempt to control for litter effects. In a subsequent experiment [16] lead acetate was administered to dams, as above, and the behavior of the offspring was compared to the behavior of rat pups receiving either $Pb(Ac)_2$ or NaAc via oral administration. The growth of the offspring in the first group was markedly depressed when compared to the growth of the other two groups, suggesting some methodological advantages to be found in the direct administration of lead to pups as opposed to their dams. In 24 hr measures of group activity, the $Pb(Ac)_2$ animals were more active than the NaAc animals. The authors were unable to replicate their previous findings concerning NE and DA concentration. Again, there was no control for the effects of litter differences. Finally, from the same laboratory, is a report [18] of the effects of lead delivered to dams in drinking water as $Pb(Ac)_2$. Animals were not pair-watered. Twelve experimental and 12 control animals were used in individual activity measures. Since animals were run individually rather than in intact litters, litter effects may have been controlled. This cannot be determined from the research report, since the manner of selection of the 12 animals is not specified. At any rate, no effects of lead on activity were found, leading the authors to speculate that the effects of lead on activity may be ". . . minimal or evanescent in nature."

The work reported above is frequently cited as evidence of the link between lead and hyperactivity in rodents and as being of relevance to the etiology of hyperactivity in children [24,44]. Reports from other laboratories using a variety of methods to expose developing rat pups to lead have yielded varying and conflicting results. Thus, when male rats were given daily oral doses of lead acetate during their first three weeks of life, they did not differ from control rats in spontaneous activity measured in a photoactometer [42]. (However, on the basis of peformance in a shuttle task and the response of the animals to amphetamine, these authors suggest that lead may be of etiological significance in MBD. Unfortunately, the data presented in this report are insufficient to evaluate this suggestion. While the authors ran enough litters per condition to evaluate this source of variance statistically, this evaluation was not done. Student's *t* was employed instead of the appropriate analysis of variance.)

Other investigators have examined the lead-behavior relationship in a variety of situations, following a variety of exposure routes. Thus, rat pups have been dosed intraperitoneally [1], dams have been injected during gestation and following parturition [41], and dams have been fed lead in diets, as described above [1], or in drinking water or by gavage [1]. The behavior of offspring has been investigated in the open field and in a perceptual discrimination task [45], in a modified Hebb-Williams maze [41], and in a T-maze. No clear pattern of lead related effects is evident in these studies.

The methodological difficulties in much of the work above have been recognized and succinctly stated by one group of workers [20]. This group has conducted one of the few methodologically sound pieces of research to be found in this area. Pair-fed control animals were used, litter effects were controlled, and appropriate statistical analyses were conducted. In contrast to many previous reports, these authors present sufficient data and statistical analyses to permit the reader to assess the appropriateness of their conclusions. These authors were unable to demonstrate any lead induced hyperactivity in their rats. Their results are at variance with the literature cited above. They question both the purported relationship between lead and hyperactivity in rats and the relevance of this animal model to childhood hyperactivity. Of some interest is the report by these authors that the lead-via-dam's food route of lead administration was not satisfactory. Dams refused to eat the adulterated food.

The authors were thus forced to intubation of dams as a technique for delivery of lead. We had similar problems in our laboratory at the outset of our work, observing refusal of adulterated food and water and some related instances of cannibalizing of litters by experimental dams.

The lack of relationship between lead exposure and hyperactivity observed by these investigators is consistent with findings in our laboratory. We have raised approximately 100 litters of Long-Evans rats in this effort. We have experimentally treated and/or dosed well over 550 of these rats in various studies of the effects of pre- and post-natal lead exposure on behavior and brain chemistry. When the appropriate experimental controls are instituted, we are unable to demonstrate a convincing relationship between such lead exposure and hyperactivity. Neither are we able to demonstrate a consistent relationship between lead and behavioral variables thought to reflect learning and/or affective states related to hyperactivity.

Some of these results have been reported elsewhere [13, 29, 30] and will be briefly summarized here. In the first phase of this work lactating dams were fed 2.0% Pb(Ac)$_2$ in powdered chow. The effects of this administration on the open field activity of the offspring and on the response of these animals to amphetamine and phenobarbital were examined. The open field was identical to that described below under Methods, except that it was painted white. Intact litters were not run. Litter effects were controlled by randomly assigning animals to drug treatments within both lead and control groups. No lead related differences in activity were found, nor were there any differences in the amount and pattern of rearing and grooming. Lead animals and control animals did not respond differentially to drug treatment. No "paradoxical" effect of amphetamine was found.

In this study we did not employ pair feeding. A comparison of pup weight, dam weight, and dam food consumption provided striking evidence for the necessity of employing pair feeding procedures when lead is administered in this way. The weight of experimental dams was significantly below that of controls throughout the pre-weaning period. Experimental dams show drastic reductions in food intake, virtually ceasing to eat upon the introduction of food containing 2.0% Pb(Ac)$_2$. As might be expected, weights of experimental pups lag behind those of controls.

In the second phase of this study, a dose-response investigation was carried out relating oral dosage of neonatal pups to brain lead concentration, activity and other behaviors in an open field, and passive avoidance behavior. Oral administration was used in an effort to increase the reliability of lead delivery to pups. This strategy was apparently effective. Over a seven dose range, from 0 mg/kg to 220 mg/kg, brain lead concentration was shown to increase monotonically with oral dose. Brain weight of pups, on the other hand, was shown to decrease monotonically with oral dose. Oral dosing of neonates proved to be a reliable means of delivering lead to experimental animals. Many of the difficulties of feeding adulterated chow are circumvented in this way.

Results of the behavioral analysis in this study are an object lesson in the need to control for litter effects. When litters are ignored as a source of variance, a significant analysis of variance treatment effect of lead is found for number of squares crossed, number of center squares crossed, and time spent in rearing. In each case the lead treated animals differ from controls in the expected direction. However, when the data are treated in a hierarchical design in which litters are treated as a source of variance, only the number of squares crossed shows a statistically reliable effect of lead. And, the linear relationship between lead and number of squares crossed is weak, lead treatment accounting for only four percent of the variance in squares crossed. Finally, there are reliable effects of litter on brain lead concentration. Thus, even though lead is directly administered to the offspring, litters are apt to differ in the amount of lead which is found in brain following a given dose.

In addition to an interest in behavioral concerns, some investigators have examined the possible relationship between lead exposure and brain chemistry. Much of this interest stems from the suggestion that dysfunction in brain amines might be involved in hyperkinetic or MBD children [43]. Conflicting reports of the effect of lead on DA and NE have been cited. Other relationships between lead and brain chemistry have been reported. Lead treated and control mice are reported to differ in their response to a variety of pharmacological compounds [36]. Significant differences in high affinity synaptosomal transport of choline, dopamine, and tyrosine have been reported by the same investigators. Other investigators have examined the effects of post-natal lead on brain RNA, DNA, DA, NE, 5-HT, and GABA (γ-aminobutyric acid), finding either no effect, or inconsistent and conflicting effects in the case of brain catecholamines [16, 21, 32].

We now report on three additional studies done in our laboratories. These involve both pre-natal, and pre-natal combined with post-natal administration of lead. In addition to measures of general activity, we present data on behaviors reflecting learning and affective states presumed relevant either to the hyperactive child syndrome or to some presumed effect of lead on the CNS. Finally, we present the effects of chronic lead administration on certain aspects of *in vivo* and *in vitro* brain chemistry.

GENERAL METHOD

Some general statements are relevant to all studies conducted in the Baylor laboratories. Animals were maintained on a 12 hr light-dark cycle, lights on at 15:00 hr (see exception under Shuttle Avoidance, Study 3, below). All behavioral testing was done between 09:00 hr and 14:00 hr during the animals' dark, or active cycle. Behavioral testing was conducted under low level, red light illumination, except in those instances where level of illumination was an independent variable. Background masking noise was provided for all behavioral tests, and, with the exception of a large apparatus such as the radial arm maze described below, each behavioral apparatus was located within an isolation cubicle. Feeding, watering, weighing, dosing, etc. of animals was done between 15:00 hr and 18:00 hr. Lead contaminated animals were housed separately from controls, in a negatively pressurized room. Cross contamination of the control colony from the atmosphere of the lead colony was therefore avoided. All animals were bred in our colony, rather than being purchased when pregnant. The desirability of this approach to developmental studies has been forcefully stated [6].

STUDY 1

Animals

All animals were Long-Evans rats bred in the Baylor animal colony. Two females and one male were housed in a plastic breeding cage for four days. On the fifth day the females were housed separately and randomly assigned to either experimental or control groups. There were 16 experimental animals and 16 control animals.

Treatment

Upon being housed separately, animals were maintained on Purina Rat Chow. The experimental females were placed on a 0.5% solution of $Pb(Ac)_2$ in lieu of normal drinking water. Control females were pair-watered each day by giving them a volume of tap water equal to the consumption of the paired experimental female on the previous day. This procedure was continued until the pups were weaned on Day 22 following parturition, parturition being designated as Day 0. Litters were culled to 8 pups (5 male and 3 female where possible) on the second day following parturition. Not all females conceived, and there were thus 10 experimental dams and 9 control dams to provide offspring for the experiments. One male pup from each of 10 experimental and one male pup from each of 8 control litters were used in behavioral testing.

Chemical Analyses

Whole brain lead was assayed when pups were 31–34 days old, using an organic solvent extraction modified in our laboratory [30] from a procedure used to assay lead in blood [46]. Brains were homogenized in 0.5% HNO_3, lead was chelated with l-pyrrolidinecarbodithioic acid (APDC) and then extracted into methyl isobutyl ketone (MIBK) for assay via a Perkin-Elmer Model 403 Atomic Absorption Spectrophotometer. Lead determinations were carried out on 9 experimental pups from 5 litters and on 11 control pups from 6 litters.

Brain calcium in hippocampus, striatum, and residual brain was analyzed by atomic emission spectrophotometry using the Perkin-Elmer Model 403. Brain dissection was by standard methods [15]. Tissue from hippocampus and from the striatum was placed in ignition tubes to which 0.2 ml concentrated nitric acid was added. The tubes were then heated on a hot plate, at a temperature not exceeding 110°C. This process of adding concentrated HNO_3 was repeated until a white ash was obtained. That is, if the initial 0.2 ml concentrated nitric acid was not sufficient to produce a white ash, another 0.2 ml was added. The residual brain was homogenized in 5.0 ml of 0.5% (v/v) HNO_3. Two 1.0 ml aliquots of the homogenate were then dried and digested as above. When the tissue was reduced to a white ash it was taken up with a solution composed of equal volumes of 0.1 N HCl and 2.0% (w/v) potassium chloride. This procedure is essentially the same as that used by other investigators [2]. The solutions so obtained were then analyzed for calcium using a Perkin-Elmer Model 403 with a nitrous oxide-acetylene flame.

Brain lead determinations at two days of age were accomplished on animals raised similarly to those above. Breeding was continued for seven days rather than four. Following breeding, experimental animals were given 0.5% $Pb(Ac)_2$ for drinking water and controls were pair-watered. On Day 3 following parturition, 6 experimental animals (2 males from each of 3 litters) and 6 control animals (2 males from each of 3 litters) were sacrificed by chloroform asphyxiation and their brains excised for lead analysis. The tissue was wet ashed in warm, concentrated HNO_3. The white ash was taken up in 0.5 ml of 0.1 N HNO_3 and neutralized with 0.5 ml of 0.1 N NaOH. Five successive 100 ml aliquots of this solution were dried in Delves Cups [5] and analyzed by atomic absorption spectrophotometry.

Radial Arm Maze

A radial arm maze was constructed which was a modification of one described earlier [27,28]. In the present case the maze contained eight arms of length 86 cm, enclosed with particle board floor 7 cm wide and particle board walls 4 cm high. The tops of the arms were made of hardware cloth. These arms were attached to an enclosed center octagon of 37 cm diameter made of particle board and fitted with a hinged top of Plexiglas. The center octagon and each arm had removable Plexiglas floors. During 12 days of testing in the radial arm maze the following procedure was used:

Days 1 through 5: The rat was placed in the center octagon and remained in the maze for at least 5 min. At the end of the 5 min period it was removed, provided it had entered each of the 8 arms at least once. The number of arm entries during this 5-min period provided a measure of activity in a novel, nonstressful situation. The number of different arms entered in the first 8 entries provided a measure of spontaneous alternation under conditions of no reward and no deprivation. Spontaneous alternation has been associated with hippocampal function [28], and lead has been shown to accumulate in the hippocampus [14]. If the animal had not entered all 8 arms at the end of the 5-min period, it was permitted to remain in the maze until it had done so or until an additional 5 min had elapsed.

On Day 5, after testing, water deprivation was begun. Both control and experimental animals were given water for only one hr per day, between 15:00 and 16:00 hr. Effectively, this resulted in a mean deprivation of 17 hr at the time of testing.

Days 6 through 10: The testing procedure described above, including deprivation, was continued except that a large drop of water was placed in the drinking cup at the end of each arm. In this way the effect of deprivation and reward on activity levels and spontaneous alternation performance could be observed.

Days 11 and 12: The procedure described above was continued except that animals were removed from the maze as soon as they had entered all 8 arms. All animals had done so within the first 5 min.

STUDY 2

Animals

All animals were Long-Evans rats bred in the Baylor animal colony. Two females and one male were housed in a breeding cage. On the fifth day of breeding cage residence, the females were removed and randomly assigned to the lead or the control group. There were 6 lead females and 6 control females. Two male offspring of each of these dams were randomly sampled for inclusion in behavioral testing. Brain chemistry was done on both male and female brains.

Treatment

Upon being housed individually, pregnant animals in the experimental group were placed on a water supply that contained 0.2% Pb, as lead acetate. The volume of water each experimental female consumed each day was determined and this volume of water was given as the next day's ration to a pair-watered control female. This procedure was continued until parturition. Beginning at birth, each litter was weighed daily and a mean pup weight determined for each litter. Lead animals were then individually administered lead acetate via the oral cavity. Dose was 225 mg of lead per kilogram of body weight (i.e. mean pup weight of the litter) administered daily in a constant volume of 0.0025 ml per gram of body weight. A 1.0 ml tuberculin syringe with needle ground smooth was used to administer the lead solution. Paired control litters were dosed in similar fashion with distilled water. At weaning, lead animals were housed as litters and given drinking water containing 0.25% lead, as lead acetate. Control litters were similarly housed and were provided with tap water in a volume determined each day by the consumption of their paired lead litter. Animals were separated by sex on Day 25 and pair-watering was continued throughout the experiment. Thus, these animals received lead prenatally and for the duration of their lives. Behavioral tasks were accomplished in the order listed in this section. All males to be used in behavioral testing were individually housed on Day 30. Behavioral testing was carried out from Day 49 to Day 58.

Biochemical Determinations

Animals were sacrificed by chloroform asphyxiation. Brain lead was assayed as in the first study by atomic absorption spectrophotometry using an organic solvent extraction for whole brain. Butanol extraction [19] was employed in the fluorometric assay of 5-Hydroxy-tryptamine (5-HT). Brains were dissected [15] into hippocampus, striatum, brain stem, and cortex, and 5-HT assayed separately in these regions. The uptake by striatal tissue minces of α-methyl-meta-tyramine (MMTA) was investigated. Minces were incubated for 30 min in a 0.2 mg/ml concentration of MMTA. Following arrest of metabolic processes at the end of this period by application of ice cold Krebs'-Ringer wash, tissue was homogenized in 0.4 N perchloric acid and extracted with a butanol:heptane mixture. The indoleamine was reacted with o-pthalaldehyde and assayed fluorometrically. The complete procedure, composition of Krebs'-Ringer, etc. is described elsewhere [7, 8, 33].

Radial Arm Maze

The radial arm maze described above was used. In Study 2 animals were run following the procedure of the previous study, except that the animals were run only one day for a five-min period. Measures of activity and of spontaneous alternation were taken as described above.

Open Field

The open field was a cube with 60 cm sides, painted flat black, and isolated from ambient noise. Animals were observed for 10 min and recording of activity was taken in Minutes 0–2, 3–5, and 6–10. Previous investigators [45] had reported differences in the open field during the first two min. This apparent effect in a novel situation was similar to a trend we had observed in the radial arm maze. The floor of the open field was inscribed with lines so as to divide it into four equal quadrants. A circle of 16 cm diameter was inscribed with center at the intersection of the quadrant lines. This circle served as a means for determining activity in the center of the open field as opposed to activity toward the periphery. Measures taken in this situation included number of squares crossed, number of center squares crossed, and total squares crossed. Ratings of behavior in this situation have reliability coefficients in the 0.90's [30]. After each animal was run, the glass floor of the maze was cleaned with a commercial isopropyl alcohol solution. Animals were run in the open field on two successive days.

Social Interaction

The amount and kind of social interaction engaged in by pairs of animals was determined in two situations which have been described as being sensitive to anxiety [10,11]. The open field box was used as one of these situations, while a similarly constructed box was used for the second. The first is designated the "familiar" box; the second is designated the "unfamiliar" box. The familiar box was painted flat black and was always used in red light illumination. The unfamiliar box was painted white and was always used in bright light illumination. The differential response of pairs of animals to these situations is well known [10, 11, 12].

The two open field trials served to familiarize each of the animals with that box. At the time of the social interaction test, each animal was placed in a box with a strange (non-litter mate) animal. Pairs of lead animals so constituted were compared with pairs of control animals. Pairs of animals were randomly assigned to either a familiar box placed in a red light illumination or to an unfamiliar box placed in a bright illumination. The amount of time these animals spent in social interaction [10,11] was scored, as were the instances of aggressive behaviors defined as wrestling, boxing, and biting.

The observer scored social interaction by activating a running-time meter whenever the animals interacted socially. The frequency of wrestling, boxing, and biting was tabulated and summed and constituted the aggression score.

Passive Avoidance

Behavior in a passive avoidance situation was investigated because of its presumed similarity to the inability of the hyperactive child to inhibit responding. The apparatus was a modification of one described elsewhere [30]. Essentially it consisted of a two-compartment box made of Plexiglas, the compartments being separated by a guillotine door that was manipulated by the experimenter. The compartments measured 42 cm by 20 cm, one being painted white, the other flat black. The floor was a grid connected to a shock source. A photoelectric beam was placed 4 mm above the floor of the border of the two compartments. Breaking this beam served to activate electronic equipment that counted the number of interruptions and recorded their duration.

Animals were placed in the white compartment facing away from the closed guillotine door. After 10 sec, the door was raised. When the animal crossed to the black side the door was dropped and a shock of 0.5 mA delivered for 1.0 sec.

The animal was removed to a holding cage for a one-min intertrial interval. This procedure was repeated until the animal did not cross to the black box for eight min following the raising of the door or until five trials had taken place. Only one animal, a control, required five trials to meet the eight-min criterion. Latency to cross was recorded on each trial.

The recording of incursions that break the photoelectric beam was done on an Esterline Angus Model AW recorder, using a paper speed of 7.6 cm/min. An incursion that occurs prior to the animal's making full entry into the black box is termed an Abortive Incursion (AI). AI's of less than 2 sec duration (brief AI's) were simply counted, to obviate observer variation in judgement of very brief time intervals. All other AI's were both counted and timed for duration of incursion. Thus, each animal yielded four measures of activity at the photoelectric beam on each trial: number of untimed incursions (brief AI's), number of timed incursions (timed AI's), total number of incursions, and mean duration of timed incursions.

STUDY 3

Animals

The animals in this study were housed and run in a separate laboratory facility. As a result, some rearing parameters were slightly changed from the procedure described earlier. The animals were maintained on a 12-hr light-dark cycle with lights coming on at 22:00 hr and going off at 10:00 hr. All behavioral testing was done during the animals' dark period, between the hours of 10:00 and 22:00. All litters were reduced to 8 pups on Day 2. Twenty male animals from 4 litters were used in behavioral testing.

Treatment

Animals were treated through parturition in the same fashion as animals in Study 2. That is, dams were maintained on lead adulterated drinking water during pregnancy (0.2% lead). Neonates were orally dosed with lead acetate at a lead dose of 90 mg/kg, from Day 1 until 21 days. At weaning on Day 21, offspring were placed on a 0.25% lead as $Pb(Ac)_2$ solution and were maintained on this solution until 33 days of age. Thereafter they received tap water ad lib. Pair-watering/pair-dosing were carried out as in Study 2.

Shuttle Activity

A standard Lehigh Valley shuttle avoidance box was placed in a sound attenuating chamber. Lighting was provided by a 7.5 W red light bulb mounted in the ceiling of the sound attenuating chamber. Background noise was provided by the blower which provided for air exchange within the chamber.

Each rat was placed in the apparatus for 15 min, and crossings across the center line were recorded for 3 successive five-min periods. Activity was measured between 30 and 33 days of age.

Shuttle Avoidance

A standard Lehigh Valley shuttle avoidance box with a 2.5 cm barrier installed was housed within a sound attenuating chamber. Scrambled shock of 0.6 mA constant current was the unconditioned stimulus (US). The conditioned stimulus (CS) was a 28 V bulb mounted in the end of the box, plus a sound (Sonalert mounted in ceiling of box). The CS came on for 5 sec, at which time the US was delivered. The CS and US then continued paired for an additional 10 sec at which time the US and CS were terminated. The animal could avoid the shock by jumping to the other side of the apparatus. The intertrial interval was 1 min. The chamber was dark, the only illumination being provided by the CS. Animals were run at age 58–60 days for 100 massed trials.

RESULTS

STUDY 1

Dam weight pre- and post-partum is shown in Fig. 1. There are no statistically reliable differences between the lead and control groups. There were no significant differences between lead and control groups in number of litters delivered, number of pups per litter, or day of eye opening of pups. There were no lead-control differences in pup weight from birth to weaning.

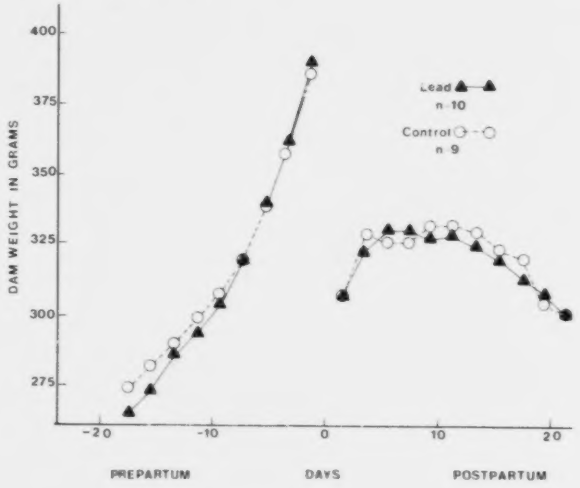

FIG. 1. Pre- and post-partum weights of lead and pair-fed control dams.

These results suggest that pair-watering is an effective control in this paradigm. There were, however, differences in brain weight. The brains of lead animals were consistently lighter than the brains of control animals. This difference is significant at Days 31–34. At this time the means and standard deviations for brain weight are, Lead: mean = 1.58 ± 0.06; Control: mean = 1.68 ± 0.06, for N of 9 and 11, respectively. This decline in brain weight consequent to lead exposure is consistent with our previous finding [13].

Chemical Analysis

At three days of age the lead animals have significantly increased concentrations of brain lead when compared to controls. There is no overlap between these groups at this age. By 3 days of age, lead animals have a mean brain lead concentration of 0.174 μg/gm of wet tissue weight while the brain lead concentration of controls is essentially zero. This difference is significant ($t = 14$, $p < 0.001$). At 30–34 days differences in brain lead concentration are not statistically significant. This phenomenon of disappearance of brain lead

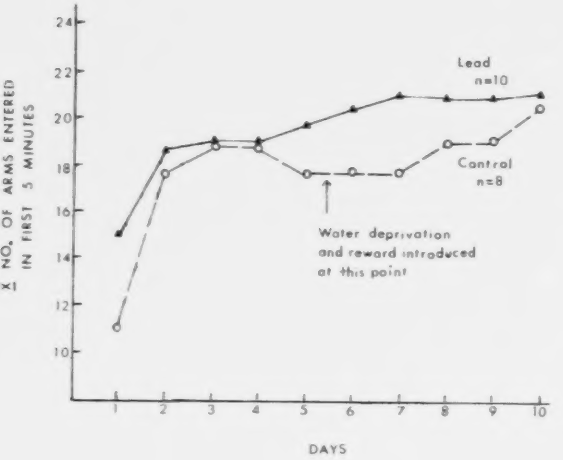

FIG. 2. Mean number of arms entered in the first five min in a radial arm maze for twelve consecutive days of testing.

following cessation of lead ingestion has been reported by others [18].

There were no differences in regional brain calcium between experimental and control groups at Days 40–45. Mean brain calcium concentrations plus and minus one SD for lead and control groups were: Hippocampus: Lead mean = 56.88 ± 6.37, Control mean = 54.56 ± 6.13; Striatum: Lead mean = 53.55 ± 6.61, Control mean = 52.92 ± 4.22 and Residual Brain: Lead mean = 52.45 ± 5.13, Control mean = 52.16 ± 3.01.

Radial Arm Maze

Figure 2 shows the mean number of arm entries in the radial arm maze in the first 5 min as a function of days. Although the experimental group was consistently more active than the control group, the overall difference did not attain statistical significance in a repeated measures analysis of variance. The apparent first day difference between lead and control animals was not replicated in our subsequent work reported below. Insofar as the number of arm entries is a measure of general activity, lead does not lead to hyperactivity in this situation.

A measure of spontaneous alternation taken in the radial arm maze failed to differentiate between lead and control animals. Mean spontaneous alternation scores as measured by the number of different arms entered in the first 8 choices plus and minus one SD are Lead mean = 5.6 ± 0.97, and Control mean = 5.63 ± 1.19 on Day 1. On Day 12 of testing, Lead mean = 6.9 ± 0.63 and Control mean = 7.13 ± 0.35.

STUDY 2

Mean pup weights from Day 3 until Day 30 following parturition are shown in Fig. 3. The groups do not differ from one another during this period. Similarly, experimental and control animals did not differ in body weight at time of testing. Lead animals were slightly lighter than control animals, but this difference is not statistically significant (Lead mean = 221.75 ± 18.57; Control mean = 226.33 ± 24.56).

Chemical Analyses

Brain lead concentrations for experimental and control offspring are shown in Table 1. These determinations are based on 6 experimental and 6 control males assayed at Days 75–76. The difference in brain lead is significant, $t(10) = 21.23$, $p < 0.001$.

Experimental and control brains did not differ in 5-HT concentrations when assayed in hippocampus, striatum, brain stem, and cortex.

MMTA uptake by striatal tissue minces did not differ in the experimental and control groups. Mean MMTA concentrations in μg/gm of tissue plus and minus one SD following a

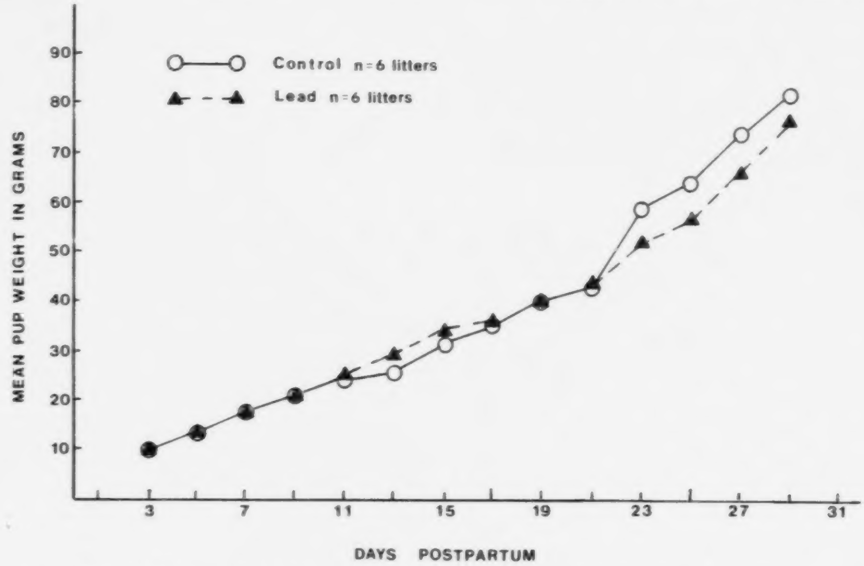

FIG. 3. Mean pup weights from birth to weaning for n=6 control and n=6 lead litters.

TABLE 1

MEAN BRAIN LEAD CONCENTRATION IN μG/GM WET TISSUE WEIGHT AT DAYS 75–76 POSTPARTUM (STUDY 2)

	Lead	Control
mean	1.85	0.13
SD	0.173	0.082
N	6	6

30-min incubation in MMTA in a concentration of 0.2 μg/ml were 0.99 ± 0.57 for experimental animals and 1.77 ± 0.38 for control animals. These means are based on 4 experimental and 3 control samples, resulting from the pooling and mincing of 3 experimental and 3 control striata.

These differences are not statistically significant, $t(5)=1.73$.

Radial Arm Maze

Lead rats did not differ from control rats in either activity or in spontaneous alternation. The mean and standard deviation for number of arm entries in a 5-min period was mean = 8.17 ± 4.62 for the lead animals and mean = 8.58 ± 4.52 for the control animals. The mean and standard deviation for number of spontaneous alternations was mean = 4.67 ± 1.80 and mean = 4.83 ± 2.23 for the lead and control groups, respectively.

Social Interaction

As expected, the familiar and the unfamiliar situations differ in the amount of social interaction they elicit. Pairs of rats interact more in the familiar situation than in the unfamiliar situation. There are, however, no statistically significant differences in those situations between lead and control groups. The means and SD for time spent in social interaction by the lead and control groups are Lead: mean = 362.23 ± 87.85 in the familiar situation, 194 ± 64.59 in the unfamiliar situation: Control: mean = 371.23 ± 59.78 in the familiar situation, 194.67 ± 28.84 in the unfamiliar situation. Previous work has shown this test to be sensitive to anxiety or fear. The above data suggest that the mean response to this situation is not different in lead and control animals.

Open Field

Figure 4 presents the number of center and peripheral squares crossed by lead and control animals on Day 1. The lead animals are reliably different from the controls with regard to peripheral squares crossed when analyzed by a 2×3 factorial analysis of variance with repeated measures on one factor. $F(1,21)=4.29$, $p<0.05$ for the main (lead) effect. $F(2,42)=5.49$, $p<0.008$ for the treatment by time-period interaction effect. Examination of Fig. 4 reveals that there are no differences between lead and control animals in Period 1 but there are differences in Periods 2 and 3. Note that the lead animals in this study are less active than the control animals, contrary to expectation.

Passive Avoidance

There were no differences between lead and control groups in trials to criterion (Lead: mean = 2.83 ± 0.687: Control: mean = 3.08 ± 0.862). On the measures of abortive entries into the dark side, however, there are interesting differences between the experimental and control groups. While lead and control animals did not differ in the mean duration of timed incursions, they differed in the number of incursions, both timed and untimed, made across trials. Figure 5 presents the mean duration of the timed incursions. Figure 6 presents the number of untimed incursions. When the number of untimed incursions is placed in ratio to the total number of incursions, the difference between experi-

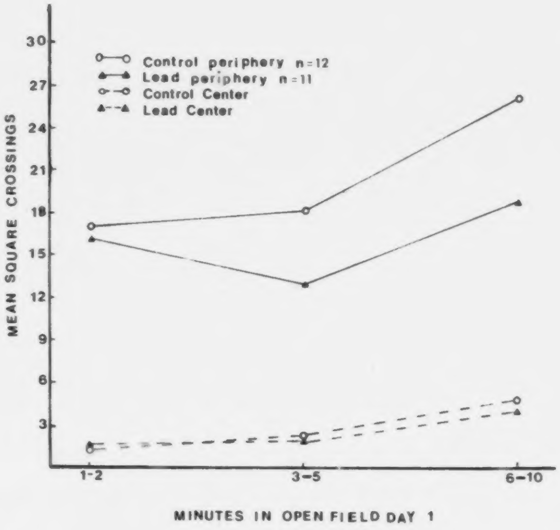

FIG. 4. Mean number of center and peripheral squares crossed by lead and control animals on Day one in the open field for Minutes 1–2, 3–5, and 6–10.

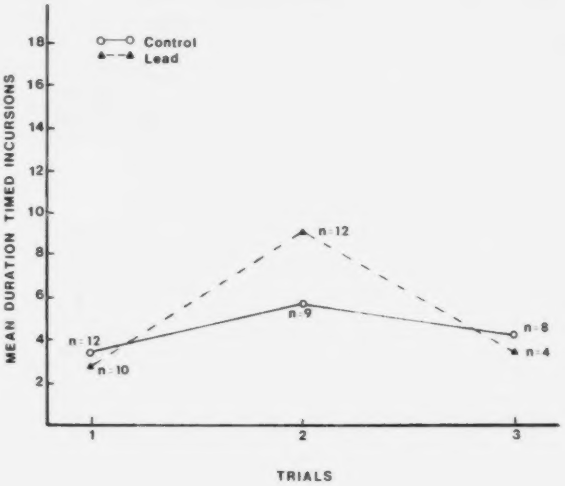

FIG. 5. Mean duration of timed incursions over trials in passive avoidance for lead and control animals.

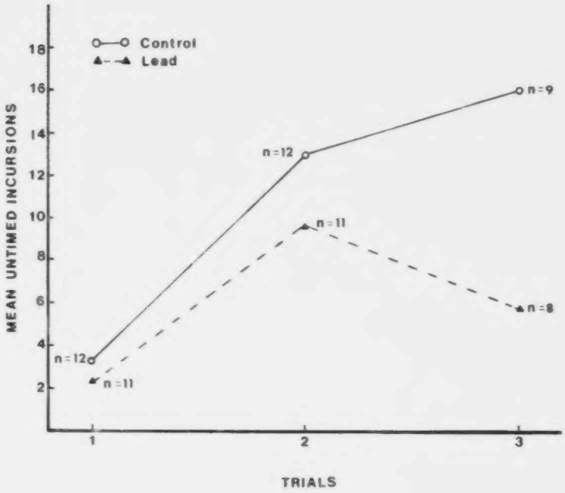

FIG. 6. Number of brief AI's over trials in passive avoidance for lead and control animals.

TABLE 2

MEAN VALUES OF THE RATIO: BRIEF AI ÷ TOTAL AI OVER TRIALS FOR LEAD AND CONTROL ANIMALS

		Trials		
		1	2	3
Lead	mean	0.5	0.67	0.633
	SD	0.289	0.208	0.215
	N	11	11	5
Control	mean	0.717	0.556	0.499
	SD	0.157	0.211	0.085
	N	12	12	8

mental and control animals is easily seen. These data are shown in Fig. 7. (n changes in these graphs because some animals reach criterion and/or show no AI's on the criterion trial). The mean values of this ratio are presented in Table 2. A repeated measures analysis of variance on these data show a significant treatment × trials effect, $F(2,32)=4.98$, $p<0.025$.

STUDY 3

There were no significant differences in weight between lead and control groups at 30–33 days when activity testing was conducted. The mean weights plus and minus one SD at this time are Lead: mean = 161.60 ± 10.70; Control: mean = 160.70 ± 7.6).

Shuttle Activity

The mean activity scores of the lead and control animals

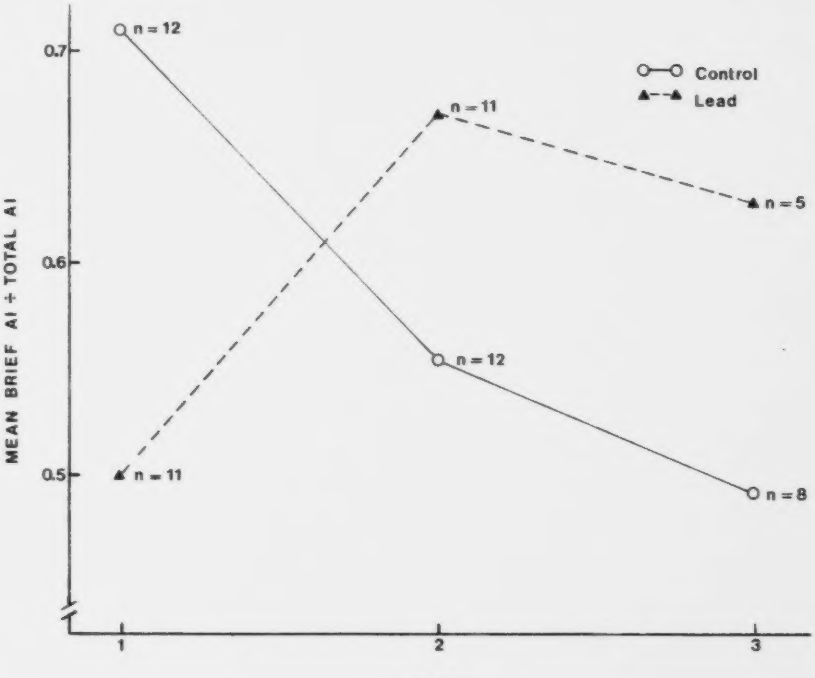

FIG. 7. Mean value of brief AI's ÷ total AI's over trials in passive avoidance for lead and control animals.

were not statistically different when analyzed in a repeated measures analysis of variance. However, the activity scores of the lead animals were somewhat lower than those of controls, a result consistent with the open field findings reported in Study 2.

Shuttle Avoidance

There were no differences between lead and control animals in number of escape or number of avoidance responses.

DISCUSSION

Inorganic lead, in the dose range considered in this paper, is not reliably associated with hyperactivity in laboratory rats. Previous reports which have suggested that lead administration early in development is productive of later hyperactivity should be re-evaluated. Methodological shortcomings and inadequate or inappropriate statistical analyses call this relationship into question. When necessary methodological constraints are imposed upon the experiment, lead is seen to bear little, if any, relationship to hyperactivity as measured in a variety of situations. In fact, lead in low doses may be unrelated to hyperactivity while higher doses (which are still small enough not to be productive of obvious symptoms of toxicity) may be productive of hypoactivity.

The behavior-related effects of lead exposure on neurochemistry remain obscure. Other investigators have reported conflicting results with respect to lead effects on catecholamines [16,30]. We have found no effect of lead on steady state DA concentrations, and no effect on regional concentrations of 5-HT. The early hopes to find a simple lead-neurotransmitter-behavior link have not been fulfilled. Research accomplished at this point in time suggests that such simple relationships are not likely to be found.

How, then, does the behavioral toxicologist proceed in the search for the effects of lead in these experimental paradigms? It will be necessary to concede at the outset that the task is not an easy one. The neurochemical organization of the brain is enormously complex, and the relationship between this complex structure and the infinite variations possible in behavior are only beginning to be understood. Recognition of the difficult nature of the tasks suggests two general strategies for further work in this area.

At the biochemical/neurochemical level, it will probably be most productive to pursue investigation into the cellular and molecular effects of lead. The effects of lead at this level are being investigated, in some cases in situations directly related to neurotransmitter systems presumed relevant to hyperactivity [38]. In this regard, attention should be directed to dynamic rather than static aspects of neurochemistry. Thus, we have found no differences in steady state calcium concentrations. Studies of uptake, release, and turnover of neurotransmitters are likely to be more informative than studies of steady state concentrations. Similarly, studies of relevant enzyme systems are indicated. Thus, lead has been shown to affect adenyl cyclase activity *in vitro* [24]. Our preliminary work along these lines has examined the effects of chronic lead exposure on striatal uptake of MMTA. We find no differences in striatal accumulation of MMTA by experimental and control animals. MMTA is taken up by striatal dopamine sensitive neurons, and the lack of effect of lead on this process argues against any very simple involvement of this system as mediator of behavioral effects of chronic exposure to lead.

At the behavioral level, it seems unlikely that a relationship is to be found between lead exposure and simple measures of activity. It is preferable to direct attention to and capitalize upon behaviors which exhibit more subtle variation than does amount of activity. One promising line of approach is that which examines qualitative activity differences in behaviors in which a large reactive component is present. The characterization of the effects of pharmacological agents on punished behavior is a good example of the fruitfulness of this approach.

We have presented evidence above of a new means of assessing such behaviors. In the repeated trials Passive Avoidance we are able to measure two behaviors that serve to quantify interesting qualitative differences between lead-exposed and control animals. The mean duration of AI's is not different in experimental and control groups. However, the lead animals show significantly fewer of these AI's than did controls. Furthermore, the number of brief AI's relative to the total number of AI's increased over trials in the lead group and decreased over trials in the control group. The picture these data present is that of the lead-exposed animals spending less time than the control in behaviors directed toward the black side of the apparatus. When the lead-exposed animal does engage in such behavior, it is increasingly tentative and increasingly associated with retreat.

One possible interpretation of these observations, of course, is one made in terms of fear, and/or punishment. It is possible that the lead-exposed aniamls are more fearful than are control animals. Or, punishment may be more disruptive of ongoing behavior in the lead-exposed animals, more readily bringing about an effect like conditioned suppression. We are currently gathering additional data on these phenomena. If these types of behavior are indeed stable under appropriate conditions, a useful measure will be available to the behavioral toxicologist or pharmacologist.

We note finally that this passive avoidance behavior is not consistent with a lead-based animal model of childhood hyperkinesis. We expected lead-exposed animals to be less able than controls to inhibit responding. They inhibit more. And, to the extent that an interpretation based on punishment and/or fear is valid, these lead-exposed animals are unlike the hyperactive child. The latter is frequently characterized as exhibiting little fear and as being unresponsive to punishment. We suggest that early exposure of rats to lead, as reviewed here, does not produce a reasonable animal model of childhood hyperkinesis.

ADDENDUM

The unexpected hypoactivity exhibited by the rats in Study 2 was surprising. A further study was undertaken in an effort to determine the underlying causes of the phenomenon. Long-Evans rats were treated as in Study 2 except that all animals, both lead and control, were given food and water ad lib one week before behavioral testing. Under these conditions the lead-control differences seen earlier failed to materialize. Mean activity scores consistently fell between those of controls and experimentals in Study 2, suggesting that two factors were operating to produce the hypoactivity observed in that study. The pair watering procedure used in Study 2 resulted in control animals who were acutely as well as chronically water deprived at the time of testing. Evidently this acute water deprivation was sufficient to elevate activity levels. The lead treated animals exhibited hypoactivity but only while they were actually ingesting lead. When lead administration was stopped, activity levels returned to normal.

REFERENCES

1. Brown, D. Neonatal lead exposure in the rat: decreased learning as a function of age and blood lead concentrations. *Toxic. appl. Pharmac.* **32:** 628–637, 1975.
2. Cardenas, H. L. and D. H. Ross. Morphine induced calcium depletion in discrete regions of rat brain. *J. Neurochem.* **24:** 487–493, 1975.
3. David, O., J. Clark and K. Voeller. Lead and hyperactivity. *Lancet.* **2:** 900–903, 1972.
4. David, O. J., S. P. Hoffman, J. Sverd, J. Clark and K. Voeller. Lead and hyperactivity—behavioral response to chelation—pilot study. *Am. J. Psychiat.* **133:** 1155–1158, 1976.
5. Delves, H. T. A micro-sampling method for the rapid determination of lead in blood by atomic absorption spectrophotometry. *Analyst* **95:** 431–438, 1970.
6. Dennenberg, V. H. Assessing the effects of early experience. In: *Methods in Psychobiology*, edited by R. E. Meyers. New York: Academic Press, 1977.
7. Dorris, R. L. and P. A. Shore. Localization and persistence of meteraminol and α-methyl-meta-tyramine in rat and rabbit brain. *J. Pharmac. expl. Ther.* **179:** 10, 1971.
8. Dorris, R. L. and P. A. Shore. Amine uptake and storage mechanisms in corpus striatum of rat and rabbit. *J. Pharmac. exp. Ther.* **179:** 15, 1971.
9. Douglas, R. J. The development of hippocampal function: implications for theory and for therapy. In: *The Hippocampus: Volume 2: Neuropsysiology and Behavior*, edited by R. I. Isaacson and K. H. Pribram. New York: Plenum Press, 1975.
10. File, S. E. and J. R. G. Hyde. The effects of p-chlorophenylalinine and ethanolamine-O-sulphate in an animal test of anxiety. *J. Pharm. Pharmac.* **29:** 735–738, 1977.
11. File, S. E. and R. R. G. Hyde. Can social interaction be used to measure anxiety? *Br. J. Pharmac.* **62:** 19–24, 1978.
12. File, S. E. Anxiety, ACTH, and 5-HT. *Trends Neurosc.* **1:** 9–11, 1978.
13. Flynn J. C., J. H. Patton and E. R. Flynn. *Oral dosage and brain lead concentration in neonatal rats: some implications for animal models of the hyperactive child syndrome.* Paper presented at the meeting of the Southwestern Psychological Association, New Orleans, April 1978.
14. Fjerdingstad, E. J., G. Danscher and E. Fjerdingstad. Hippocampus: selective concentration of lead in the normal rat brain. *Brain Res.* **80:** 350–354, 1974.
15. Glowinski, J. and L. L. Iverson. Regional studies of catecholamines in the rat brain. *J. Neurochem.* **13:** 655–669, 1966.
16. Golter, M. and O. Michaelson. Growth, behavior, and brain catecholamines in lead-exposed neonatal rats: a reappraisal. *Science* **187:** 359–361, 1975.
17. Gross, M. and W. Wilson. *Minimal Brain Dysfunction.* New York: Brunner Mazel, 1974.
18. Hastings, L., G. P. Cooper, R. L. Bornschein and I. A. Michaelson. Behavioral effects of low level neonatal lead exposure. *Pharmac. Biochem. Behav.* **7:** 37–42, 1977.
19. Jacobowitz, D. M. and J. S. Richardson. Method for the rapid determination of norepinephrine, dopamine, and serotonin in the same brain region. *Pharmac. Biochem. Behav.* **8:** 515–519, 1978.
20. Krehbiel, D., G. A. Davis, L. M. LeRoy and R. E. Bowman. Absence of hyperactivity in lead-exposed developing rats. *Envir. Hlth. Perspec.* **18:** 147–157, 1976.
21. Michaelson, I. Effects of inorganic lead on RNA, DNA, and protein content in the developing neonatal rat brain. *Toxic. appl. Pharmac.* **26:** 539–548, 1973.
22. Michaelson, I. and M. Sauerhoff. An improved model of lead-induced brain dysfunction in the suckling rat. *Toxic. appl. Pharmac.* **28:** 88–96, 1974.
23. Michaelson, I. and M. Sauerhoff. Animal models of human disease: severe and mild lead encephalopathy in the neonatal rat. *Envir. Hlth. Perspec.* **28:** 88–96, 1974.
24. Nathanson, J. A. and F E. Bloom. Lead induced inhibition of brain adenyl cyclase. *Nature* **255:** 419–420, 1975.
25. Niklowitz, W. J. and D. W. Yeager. Interference of Pb with essential brain tissue Cu, Fe, and Zn as main determinant in experimental tetraethylead encephalopathy. *Life Sci.* **13:** 897–905, 1973.
26. Niklowitz, W. J. Subcellular mechanisms in lead toxicity: significance in childhood encephalopathy, neurological sequelae, and late dementias. In: *Neurotoxicology*, edited by Roizin, H. Shiraki and N. Grevic. New York: Raven Press, 1977.
27. Olton, D. S. and R. J. Samuelson. Remembrance of places past: spatial memory in rats. *J. exp. Psychol.: Animal Behav. Proc.* **2:** 97–116, 1976.
28. Olton, D. Spatial memory. *Scient. Am.* **236:** 82–98, 1977.
29. Patton, J. H., J. C. Flynn and E. R. Flynn. *The effect of inorganic lead (Pb) on the behavioral activity of young rats in open field and passive avoidance situations.* Paper presented at the meeting of the Southwestern Psychological Association, New Orleans, April 1978.
30. Patton, J. H. *The behavior and physiological effects of administering lead to neonatal rats: a test of the model of childhood hyperactivity.* Unpublished doctoral dissertation, Baylor University, 1978.
31. Pihl, R. O. and M. Parkes. Hair element content in learning disabled children. *Science* **198:** 204–206, 1977.
32. Sauerhoff, M. W. and I. Michaelson. Hyperactivity and brain catecholamines in lead-exposed developing rats. *Science* **182:** 1022–1024, 1973.
33. Shore, P. A. and H. S. Alpers. Fluorometric estimation of meteraminal and related compounds. *Life Sci.* **3:** 551–554, 1964.
34. Silbergeld, E. and A. Goldberg. A lead induced behavior disorder. *Life Sci.* **13:** 1275–1283, 1973.
35. Silbergeld, E. and A. Goldberg. A lead-induced behavioral dysfunction: an animal model of hyperactivity. *Expl Neurol.* **42:** 146–157, 1974.
36. Silbergeld, E. K. and A. M. Goldberg. Pharmacological and neurochemical investigations of lead-induced hyperactivity. *Neuropharmacology* **14:** 431–444, 1975.
37. Silbergeld, E. K. Interactions of lead and calcium on synaptosomal uptake of choline and dopamine. *Life Sci.* **20:** 309–318, 1977.
38. Silbergeld, E. K. and J. J. Chisolm. Lead poisoning: altered urinary catecholamine metabolites as indicators of intoxication in mice and children. *Science* **192:** 153–155, 1976.
39. Slob, A., C. Snow and E. de Natris-Mathot. Absence of behavioral deficits following neonatal undernutrition in the rat. *Devl Psychobiol.* **6:** 177–186, 1973.
40. Smart, J. Activity and exploratory behavior of adult offspring of undernourished rats. *Devl Psychobiol.* **7:** 315–321, 1974.
41. Snowdon, C. Learning deficits in lead-injected rats. *Pharmac. Biochem. Behav.* **1:** 599–603, 1973.
42. Sobotka, T. J. and M. Cook. Postnatal lead acetate exposure in rats: possible relationship to minimal brain dysfunction. *Am. J. ment. Defic.* **79:** 5–9, 1974.
43. Wender, P. H. *Minimal Brain Dysfunction in Children.* New York: Wiley, 1971.
44. Wessel, M. A. and A. Dominski. Our children's daily lead. *Am. Scientist* **65:** 294–298, 1977.
45. Winneke, G., A. Brockhaus and R. Baltissen. Neurobehavioral and systemic effects of longterm blood lead elevation in rats. *Archs Toxic.* **37:** 247–263, 1977.
46. Zinterhoffer, L., P. Jatlow and A. Fappiano. Atomic absorption determination of lead in blood and urine in the presence of EDTA. *J. Lab. clin. Med.* **78:** 664–674, 1971.

Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function
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Performance and Acquisition of Serial Position Sequences by Pigeons as Measures of Behavioral Toxicity

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MCMILLAN, D. E. *Performance and acquisition of serial position sequences by pigeons as measures of behavioral toxicity.* NEUROBEHAV. TOXICOL. **1:** Suppl. 1, 105–111, 1979.—A procedure has been developed to measure the repeated acquisition of serial position sequences and to study the effects of drugs and toxic chemicals on the behavior generated by the procedure. Thus far experiments using the procedure have shown: (1) Performance schedules generate lower error rates than corresponding acquisition schedules: (2) Addition of a reset contingency further decreases errors under both performance and acquisition schedules: (3) Chained acquisition and performance schedules generate lower error rates than corresponding tandem acquisition and performance schedules: (4) Chained acquisition and performance schedules produce behavior that usually is more sensitive to drugs than corresponding tandem acquisition and performance schedules: (5) Acquisition schedules produce behavior that usually is more sensitive to drugs than corresponding performance schedules: and (6) Lead is an exception in that it produced clearer effects under a chained performance schedule with a reset contingency than under a corresponding acquisition schedule. The greater sensitivity to drug effects of behavior under acquisition schedules than behavior under performance schedules and of behavior under chained schedules may be a function of the baseline error rates, rather than the behavioral processes of acquisition, performance, and stimulus control.

Behavioral toxicity   Serial position sequences   Acquisition   Performance

EFFECTS OF CHEMICALS ON ACQUISITION AND PERFORMANCE

There is a growing recognition that a complete assessment of the possible toxic effects of a chemical must include a determination of the effects of that chemical on behavior in addition to the more traditional indices of toxicity [7, 11, 12, 13, 15]. One important aspect of behavior that might be affected by a toxic chemical is the acquisition of new behavior. Unfortunately, it is very difficult to study the effects of chemicals on the acquisition of new behaviors because problems arise that are usually not encountered during studies of the effects of chemicals on well established performance.

In order to study the effects of a chemical on established performance, it has been customary to develop stable baseline patterns of responding. Frequently, stable baselines have been developed by food depriving the animal and then conditioning the animal to make a specified response, such as a lever press or a key peck, in order to produce food. Once behavior has stabilized under a particular schedule describing the relationship between food delivery and responding, toxic chemicals are administered, either on an acute or a chronic basis. This procedure allows the investigator to study the effects of chemicals on the performance of individual animals because the behavior is reproducible from day to day and changes in behavior after administration of the chemical can be assumed to have been caused by the chemical. In addition, intersubject comparisions usually can be made, since all subjects have had similar training experiences and their stable baseline performances are similar although not identical. Some examples of the use of this approach to study the effects of toxic chemicals on behavior would include the work of our group with lead (Barthalmus *et al.*, [2]) and the work of the Rochester group with mercury (Evans *et al.*, [6]).

It is much more difficult to study the effects of toxic chemicals on the acquisition of new behaviors. The behavioral history of some organisms may favor the acquisition of new behaviors more than does the behavioral history of other organisms, so that all organisms do not begin to acquire new behaviors from a common baseline. Furthermore, as acquisition proceeds, the behavior baseline is constantly changing, perhaps at different rates in different organisms and perhaps irreversibly. These factors make it almost impossible to make repeated measurements of the effects of chemicals on the acquisition of behavior in individual subjects and intersubject comparisions are often limited because of the high degree of variability across subjects. Under such circumstances, it may be impossible to detect subtle effects of toxic chemicals.

REPEATED ACQUISITION OF SERIAL POSITION SEQUENCES

Some of these problems have been solved by the development of a technique for measuring the repeated acquisition of serial position sequences [3, 4, 14]. In these experiments monkeys were trained to respond on different levers in a predetermined sequence to obtain food. Responses on a

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lever that were out of sequence (errors) produced a timeout from reinforcement. Completion of the correct sequence of responses on the different levers produced food. By presenting the monkey with a new sequence to be acquired each session, it was possible to measure acquisition repeatedly in a single subject.

Thompson [16,17] modified this procedure to study the repeated acquisition of position sequences by pigeons. Under a chained schedule, pigeons responded in a chamber with three response keys, all of which were illuminated at the same time with one of four different colors. Pecking the correct key changed the color of all three keys to another color. In contrast, under a tandem schedule, the keys were always illuminated with one color which did not change after a correct response. Under both chained and tandem schedules, incorrect responses (responses out of sequence, or errors) produced a timeout period of total darkness in the chamber during which responses had no programmed consequences. The completion of the four-response sequence operated the feeder for a period that was too short to allow the pigeon time to eat, but after the response sequence had been completed five times, the feeder operated for a period long enough to allow the pigeon to eat. If the same problem is presented to the pigeon each day, the bird's behavior may be considered as a performance baseline, but if the sequence is changed every day, acquisition can be measured repeatedly in a single subject. Thompson found that after a few months of training, the pigeon learned a new sequence each day without wide variation in the number of errors.

In our laboratory, we have modified Thompson's procedure by introducing a contigency whereby each peck on an incorrect key resets the sequence to the beginning step [8]. Pigeons were trained in a chamber containing three response keys to peck the center response key which was transilluminated with a blue light. The two side keys remained dark and responses on these keys had no programmed consequences. Once responding on the center key was established, the key transilluminated with the blue light changed position after each food delivery. Only a response on a lighted key produced food (stage 1, Fig. 1). Subsequently, all three keys were transilluminated with a red light, and a response on any one of the three red keys changed the color to blue, but only a peck on one predetermined blue key produced food (stage 2, Fig. 1). Pecking on either of the other blue keys produced a timeout. If a reset contingency was to be used, it was introduced during the same session as the timeout. The chain was extended in a similar manner to include a green light (stage 3, Fig. 1). Responses on one of the green keys changed the color of all three keys to red, while responses on the other green keys produced a timeout. If the correct green key was pecked and all three keys became red, a response on one red key changed the color of all three keys to blue, while responses on the other red key produced a timeout (and reset the sequence to the first step where all keys were green if a reset contingency was in effect). If the correct red key was pecked and all three keys became blue, a response on one blue key produced food, while responses on the other blue keys produced timeout (and reset the sequences to the first step where all keys were green if a reset contingency was in effect). Finally, the chain was extended in a similar manner to include a yellow key light (stage 4, Fig. 1). After about 25–40 sessions, some of the birds were switched to a tandem schedule where the key colors did not change following correct responses.

The repeated acquisition of serial position sequences in these birds under chained and tandem schedules with a reset contingency was compared to the repeated acquisition of birds under chained and tandem schedules without a reset contingency. Addition of the reset contingency reduced the total number of errors made during a session under both chained and tandem schedules of repeated acquisition as shown in Fig. 2. Furthermore, errors were lower under the chained schedules than under the corresponding tandem schedules.

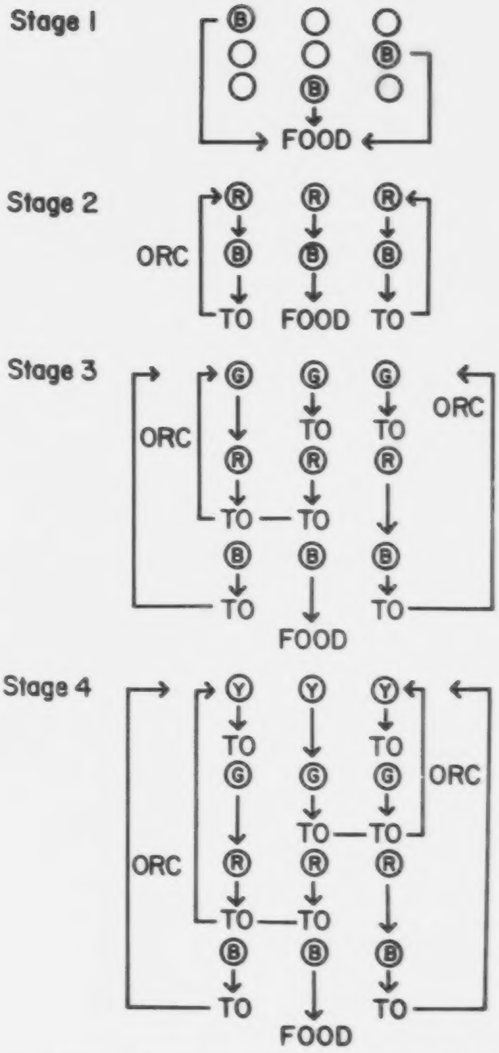

FIG. 1. Diagram of method for training pigeons to perform serial position sequences, with or without a reset contingency. Abbreviations are as follows: ○, dark key; Ⓑ, blue key; Ⓡ, red key; Ⓖ, green key; Ⓨ, yellow key; TO, timeout; ORC, optional reset contingency. Other details are in the text.

EFFECTS OF DRUGS ON RESPONDING UNDER ACQUISITION AND PERFORMANCE SCHEDULES

Thompson [16–22] has used chained and tandem acquisition and performance schedules (without a reset contin-

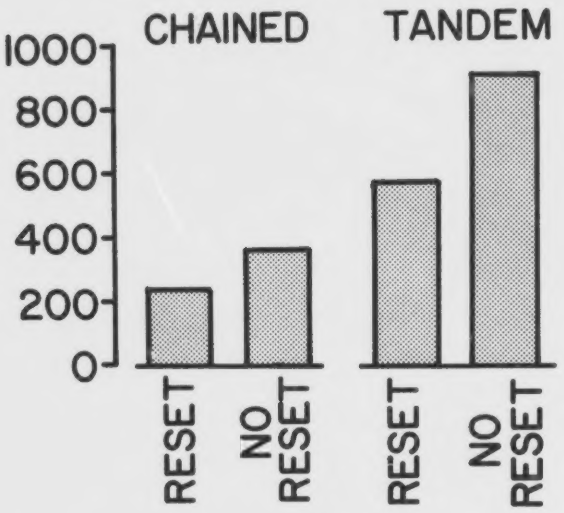

FIG. 2. Differences in total number of errors under chained and tandem schedules with and without reset contingencies. All bars are based on five sessions for three pigeons after several months of training. (Redrawn from Harting and McMillan [8])

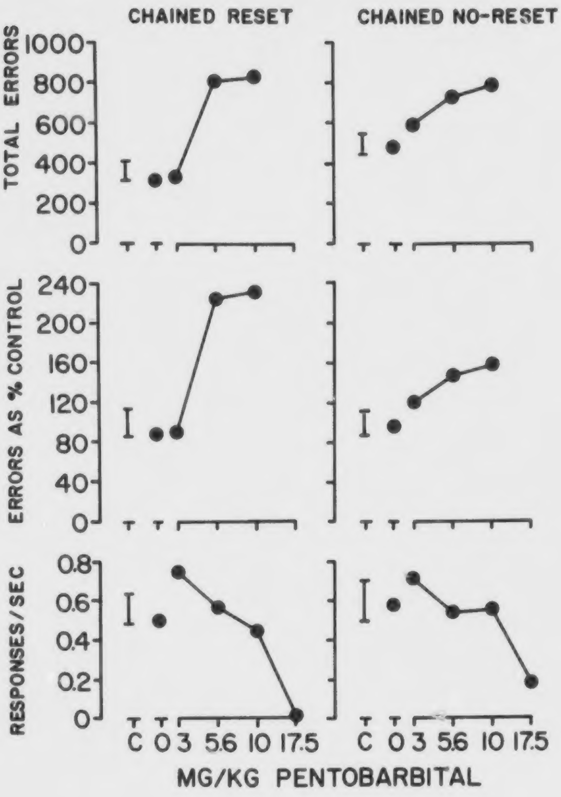

FIG. 3. Effects of pentobarbital on total errors (top row), errors as a percentage of the control errors (middle row), and mean rate of responding (bottom row) for birds under the chained acquisition schedule with a reset contingency and for birds under the chained acquisition schedule without a reset contingency. The brackets at C show ±2 standard errors of the mean for 6 control sessions in each of 3 birds. The points at zero are duplicate observations of the effects of saline in these birds. Points were not plotted after 17.5 mg/kg pentobarbital in some instances because this dose almost completely eliminated responding. (Redrawn from Harting and McMillan [9])

gency) to study the effects of drugs. Phenobarbital, chlordiazepoxide, *d*-amphetamine, cocaine, imipramine, and methylphenidate all increased errors under these schedules, but fenfluramine and chlorpromazine did not increase errors even at doses that decreased rates of key pecking [18–22]. The chained acquisition schedule was more sensitive to the effects of drugs than was the corresponding tandem acquisition schedule [22]. Furthermore, behavior under the chained acquisition schedule frequently was affected by lower doses of drugs than was behavior under the chained performance schedule [22]. Thus, the chained acquisition schedule appeared to be the most sensitive schedule that Thompson used to measure drug effects.

In our laboratory we have found that errors under the chained acquisition schedule with a reset contingency are increased to a greater extent than errors under this schedule without a reset contingency [9]. Pigeons trained under the chained acquisition schedule either with or without a reset contingency were given various doses of pentobarbital. The effects of pentobarbital on behavior under these schedules is shown in Fig. 3. In Fig. 3 errors have been plotted both as absolute number of errors and as a percentage of the control errors so that both absolute and relative error increases can be compared across the schedules.

Fig. 3 shows that pentobarbital produced greater increases in the total errors per session under the chained schedule when the reset contingency was in effect, both when errors are considered in terms of absolute increases (top row) and in terms of a percentage of control errors (middle row). These increases in errors were independent of effects on rate of responding (bottom row). Both with and without the reset contingency in effect, the largest increases in errors occurred after the 5.6 and 10 mg/kg doses, yet average rates of key pecking were little affected at these doses.

Thompson [22] also has found behavior under chained acquisition schedules to be affected by lower doses of drugs than behavior under chained performance schedules where the pigeons are exposed to the same serial position sequence every day. Our data [1] suggest that behavior under a chained acquisition schedule with a reset contingency also is affected by lower doses of drugs than behavior under a performance schedule with a reset contingency. Figure 4 shows the effects of ethanol and diazepam on responding under these acquisition and performance schedules. Again, increases in errors have been plotted both on an absolute scale and as a percentage of control errors.

Figure 4 shows that diazepam increased errors under the acquisition schedule (marked A in the figure) at a dose of 1 mg/kg, but 3 mg/kg of diazepam were required to increase errors under the performance schedule (marked P in the figure). Furthermore, absolute increases in errors after diazepam were much larger under the acquisition schedule. Similarly, ethanol increased errors at lower doses and by a larger absolute amount under the acquisiton schedule than

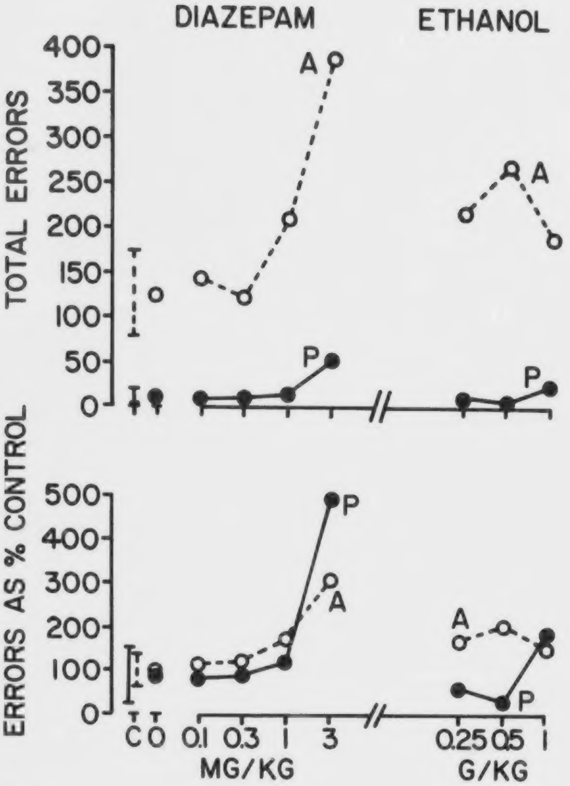

FIG. 4. Effects of diazepam and ethanol on responding under performance (P) and acquisition (A) schedules with a reset contingency. The top row shows total errors and the bottom row shows total errors as a percentage of control errors. Brackets at C show the range around the mean for at least 7 control sessions. Points at zero show the effects of vehicle administration. Diazepam was administered intramuscularly and ethanol by oral intubation. The dotted lines and unfilled circles marked A show data under the acquisition schedule and the solid lines and filled circles marked P show data under the performance schedule. (Redrawn from Barthalmus *et al.* [2])

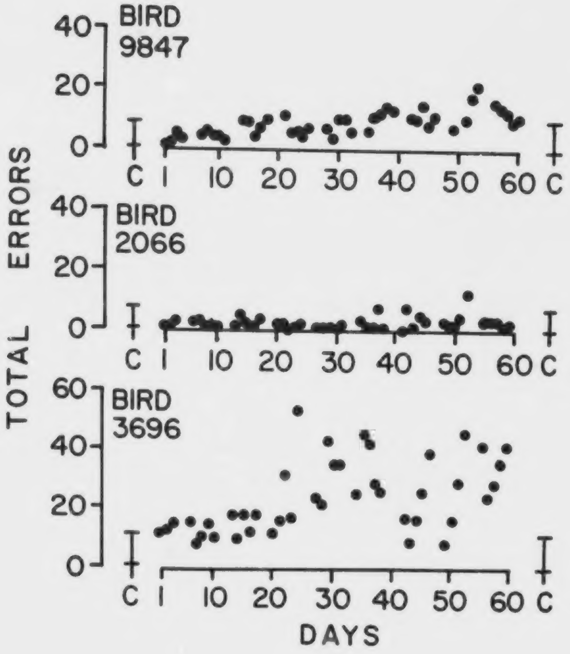

FIG. 5. Total errors for individual birds under the chained performance schedule with a reset contingency. Brackets at C show ±2 standard deviations around the control mean based on at least 15 sessions before the beginning of lead administration. (Redrawn from Dietz and McMillan [5])

under the performance schedule. Although both drugs increased errors under the acquistion schedule at lower doses than under the performance schedule, the relative increases in errors (errors as percent of control errors, bottom row) were as great or greater under the performance schedule.

EFFECTS OF LEAD ON RESPONDING UNDER ACQUISITION AND PERFORMANCE SCHEDULES

Recently we have used chained acquisition and performance schedules with a reset contingency to study the effects of the chronic administration of lead in pigeons [5]. In previous experiments in our laboratory [2] we had found that daily oral doses of lead acetate as low as 12.5 mg/kg had changed rates of responding in adult pigeons whose performance was maintained under a multiple fixed-ratio fixed-interval schedule of food presentation; however, a 6.25 mg/kg dose produced little or no effect. Therefore, the 6.25 mg/kg dose was administered chronically to pigeons under the performance and acquisition schedules in order to determine if these schedules could detect behavioral effects of lead.

Figure 5 show the effects of chronic oral doses of 6.25 mg/kg of lead on the total number of errors made during approximately two months of lead administration under the chain performance schedule with a reset contingency. Bird 9847 shows no effect for about 5 weeks of lead administration after which a clear increase in the number of performance errors occurred. Bird 2066 showed a marginal increase in the number of errors, beginning at about the same time. In contrast, lead produced an almost immediate increase in error rate in bird 3696 which had peaked by about the third week of lead administration. Thus, chronic lead administraion clearly increased errors under the performance schedule. In general, these increases in errors occurred without changes in the rate of key pecking. Following the discontinuation of lead administration, the error rate gradually returned toward the baseline error rate over a period of 6 to 10 weeks.

Figure 6 shows the effects of the same 6.25 mg/kg oral dose of lead on the total number of errors made by three birds under the chain acquisition schedule with a reset contingency during approximately two months of lead administration. None of the birds showed consistent error increases. Bird 6885 did show increases in errors on days 29 and 49. Interestingly, during both of these sessions the correct sequence was center key, right key, left key, right key.

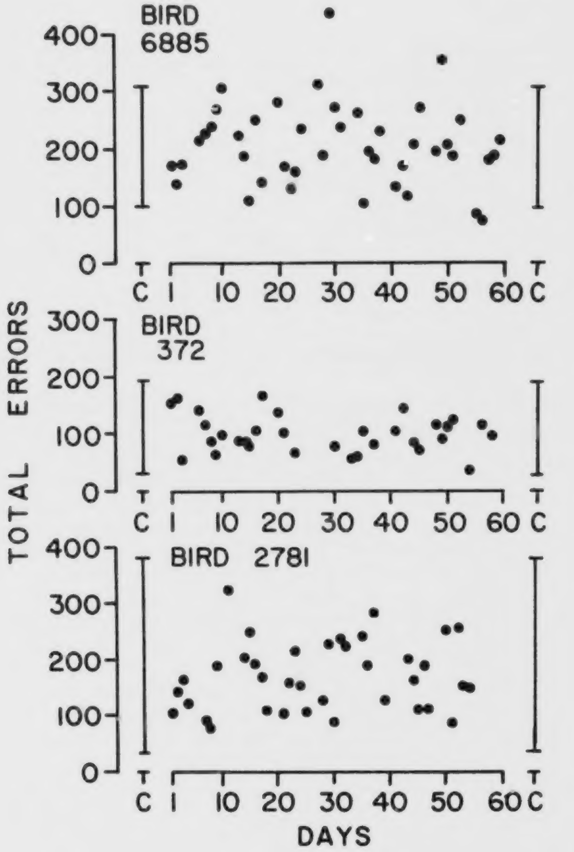

FIG. 6. Total errors for individual birds under the chained acquisition schedule with a reset contingency. Brackets at C show ±2 standard deviations around the control mean based on at least 15 sessions before the beginning of lead administration. (Redrawn from Dietz and McMillan [5])

Prior to exposure to lead, this particular sequence did not generate an especially large number of errors. Why this particular problem apparently interacted with chronic lead administration to increase errors while other sequences did not, is unclear. At any rate, the chained acquisition with a reset contingency appeared to be much *less* sensitive to the effects of a 6.25 mg/kg dose of inorganic lead than did the corresponding performance schedule.

The finding that behavior under the performance schedule was affected at a lower dose of lead than behavior under the acquisition schedule contrasts sharply with the findings of Thompson and with previous observations in our own laboratory. Behavior generated by acquisition schedules, whether or not a reset contingency was involved, has been affected at lower doses of all drugs studied than has behavior generated by peformance schedules. Lead appears to have exactly the opposite effect, that is it increases error under the chained peformance schedule with a reset contigency at a dose that has little effect on errors under the corresponding acquisition schedule.

The data in Fig. 5 and 6 were derived from pigeons whose behavioral session terminated after 600 correct responses had been made. This made it possible to study the within-session error pattern by dividing each session into six blocks of 100 correct responses and plotting the number of errors made while completing each block of 100 correct responses. Figure 7 shows that under the performance schedule (column 1) the number of errors made during each block was relatively constant prior to lead exposure. Increases in errors produced by lead tended to be concentrated at the beginning of the session, especially for bird 9847. During the final block of 100 correct responses, neither bird made more errors under lead than under control conditions. Thus, for both birds the increase in errors produced by lead had been eliminated by the end of the session. Whether this elimination of errors by the end of these sessions represents a behavioral adaptation to the effect of lead, or is merely an artifact produced by the increase in control variability toward the end of control sessions prior to lead exposure, cannot be answered.

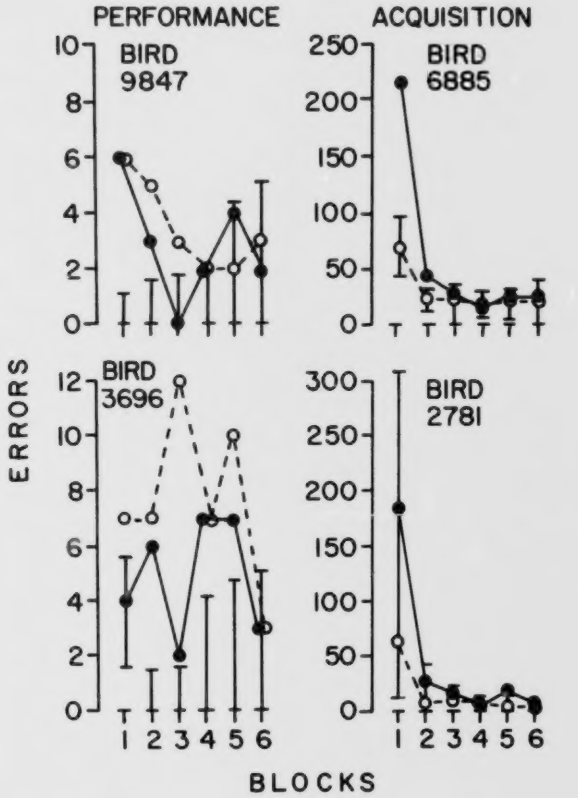

FIG. 7. Within session error elimination for individual birds under the chained performance (column 1) and chained acquisition (column 2) schedules with a reset contingency. The abscissa is blocks of 100 correct responses and the ordinate is the number of errors in each block. The brackets show ±2 standard deviations around a control mean (mean not shown) based on the last 5 sessions prior to lead administration. The sessions plotted are two successive sessions after about 7 weeks of lead administration. (Redrawn from Dietz and McMillan [5])

Figure 7 also shows the within-session error elimination before and during lead administration for the chain acquisition schedule (column 2) with a reset contingency. For bird 6885, session 48 was chosen (filled points) because of the unusually high error rate during this session. Figure 7 shows that almost all of this error increase occurred during the first block of 100 correct responses, where most of the acquisition errors were occurring. During the next session, errors were at a normal level in every block. The within-session pattern of error reduction was normal during both lead sessions that are shown for bird 2781.

The control variability was very high for bird 2781 in the first block of 100 correct responses, after which the number of errors decreased and became much less variable. The variability in day to day errors, especially during the first block of 100 correct responses under the chained acquisition schedule with a reset contingency, is probably largely a function of sequence difficulty. The acquisition of some sequences appears to be more difficult for the birds than is the acquisition of other sequences. For example, the number of errors under control conditions is outlined in Table 1 for selected sequences for some of the birds studied by Dietz and McMillan [5]. It is obvious that the right, left, center, right, and the right, center, left, center sequences consistently generated a much higher number of errors than did the center, left, right, center, and the left, center, left, right sequences. Although variability due to differences in problem difficulty may mask subtle effects of lead, it should be emphasized that this chained acquisiton schedule with a reset contingency produced behavior that was sensitive to the effects of a variety of drugs despite the baseline variability (Fig. 3 and 4). In fact, this acquisition schedule produces behavior that is generally sensitive to lower doses of drugs than is behavior under the corresponding performance schedule.

TABLE 1

AVERAGE NUMBER OF ERRORS FOR ALL BIRDS DURING REPEATED ACQUISITION OF FOUR DIFFERENT SEQUENCES*

Sequence	Mean Total Errors	Standard Error	Number of Times the Sequence Was Studied
RLCR	285	35	10
RCLC	226	39	9
CLRC	104	14	11
LCLR	127	20	9

R = right; C = center; L = left
*Data from unpublished observations by Dietz and McMillan

TABLE 2

ERRORS BEFORE, DURING, AND AFTER LEAD ADMINISTRATION IN BIRD 2654 UNDER THE CHAINED ACQUISITION SCHEDULE WITH A RESET CONTINGENCY*

Condition	Sessions	Errors (Mean ± SEM)	% Errors in 1st Bin
Control	28	230.2 ± 18.8	73.1
Lead	32	183.8 ± 15.0	63.9
Control	23	170.3 ± 13.9	55.3
Lead	35	131.4 ± 7.9	52.6
Control	71	112.3 ± 4.3	57.5

*Data from unpublished observations by Dietz and McMillan.

Thompson (1978) has emphasized that after extended training under acquisiton schedules without reset contingency, a steady state is reached so that the total number of errors per session stays relatively constant. Although most of our early data supported the idea that a steady state was achieved after extended training under acquisition schedules with a reset contingency, a problem arose in data interpretation when the effects of a prolonged period of lead administration were studied on behavior under this schedule. This problem is illustrated in Table 2 where data are shown for bird 2654 after almost two years of training under the chained acquisition schedule with a reset contingency. When the first 28 control sessions are compared to the 32 sessions under lead, the data suggest that lead administration might be decreasing the error rate during acquisition, since there was an apparent reduction in errors during chronic lead administration. Fortunately, lead administration was discontinued in this pigeon, reinstituted and then discontinued again. During each of these manipulations, the average number of errors continued to decrease further. Thus it appears that even after years of extended training under this schedule, the baseline is very slowly changing in the direction of further error elimination. This change is so slow that during the determination of drug dose-effect curves over a few weeks time, the baseline probably can be considered to represent a steady state, but during long term chronic administration of drugs or toxic chemicals these slow baseline changes could lead to misinterpretations of the data, especially if the effects of the toxic chemical are irreversible. Of course, if the behavioral effects of the chemical are large with respect to the slow change in baseline, this will not be a serious problem.

In the study by Dietz and McMillan [5] it is possible that the very gradual decrease in the error baseline over long periods of time might be exerting an influence in a direction opposite to an increase in errors produced by lead, so that any small increases in errors produced by lead during repeated acquisition would be cancelled. We attempted to evaluate this possibility by using a statistic called the split middle method of trend estimation [10]. This statistic provides an estimate of trend over time. In the case of our experiments, the null hypothesis is that there is no change in the pre-lead baseline error trend during the period of lead administration. Using the split middle trend analysis, we were able to show that errors by some pigeons under the acquisition schedule were significantly increased above the number predicted by the trend of pre-exposure errors. Whether this really is an effect of lead, or is an effect caused by some other factor controlling the trend of error elimination, remains uncertain.

SUMMARY AND CONCLUSION

It is tempting to suggest that the behavioral processes underlying repeated acquisition are more sensitive to drug effects than are the behavioral processes underlying performance, while the reverse is true for lead. However, it is dangerous to interpret these data in terms of differential sensitivity of performance and acquisition. As we have discussed previously [5], it may be the baseline error rate that determines the sensitivity of the behavioral baseline to chemicals. The performance schedules generally result in fewer errors than do similar acquisition schedules. If a very simple acquisition schedule is used, so that few errors are generated, and a complex performance schedule is used, so that many errors are generated, the effects of chemical intervention may be quite different from those recorded thus far. Until experiments are performed to determine the effects of drugs and toxic chemicals on acquisition and performance baselines that have been equalized with respect to error rates, it will be inappropriate to make general statements about the relative sensitivities of these behavioral processes.

Nevertheless, lead does appear to be unique among chemicals that have been studied with present methods. All other chemicals that have produced increases in total errors under these acquisition and performance baselines have done so at lower doses under the higher error-rate acquisition baselines than under the lower error-rate performance baselines. In contrast, a chronic dose of 6.25 mg/kg/day of lead produced clear increases in errors under the performance schedule, but it was difficult to show that this dose of lead was increasing errors under the acquisition schedule.

REFERENCES

1. Barthalmus, G. T., J. D. Leander and D. E. McMillan. Combined effects of ethanol and diazepam on performance and acquisition of serial position sequences by pigeons. *Psychopharmacology* **59**: 101–102, 1978.
2. Barthalmus, G. T., J. D. Leander, D. E. McMillan, P. Mushak and M. R. Krigman. Chronic effects of lead on schedule-controlled pigeon behavior. *Toxic. appl. Pharmac.* **42**: 271–284, 1977.
3. Boren, J. J. Repeated acquisition of behavioral chains. *Am. Psychol.* **17**: 421, 1963 (abstract).
4. Boren, J. J. and D. D. Devine. The repeated acquisition of behavioral chains. *J. exp. analysis behav.* **11**: 651-660, 1968.
5. Dietz, D. D. and D. E. McMillan. Effects of chronic lead administration on acquisition and performance of serial position sequences by pigeons. *Toxic appl. Pharmac.* **47**: 377–384, 1979.
6. Evans, H. L., V. G. Laties and B. Weiss. Behavioral effects of mercury and methylmercury. *In:Contemporary Research in Behavioral Pharmacology*,edited by D. E. Blackman and D. J. Sanger. New York: Plenum Press, 1978, pp. 207–224.
7. Evans, H. L. and B. Weiss. Behavioral toxicology. In: *Contemporary Research in Behavioral Pharmacology*, edited by D. E. Blackman and D. J. Sanger. New York: Plenum Press, 1978, pp. 449–487.
8. Harting J. and D. E. McMillan. Repeated acquisition of response sequences by pigeons under chained and tandem schedules with reset and non-reset contingencies. *Psychol. Rec.* **26**: 361–367, 1976.
9. Harting, J. and D. E. McMillan. Effects of pentobarbital and *d*-amphetamine on the repeated acquisition of response sequences by pigeons. *Psychopharmacology* **49**: 245–248, 1976.
10. Kayden, A. E. Statistical analyses for single-case experimental designs. In: *Single-case Experimental Designs: Strategies for Studying Behavior Change*, edited by M. Hersen and D. H. Barlow, New York: Pergamon Press, 1976, pp. 265–316.
11. Laties, V. G., P. B. Dews, D. E. McMillan and S. E. Norton. Behavioral toxicity tests. In: *Principles and Procedures for Evaluating the Toxicity of Household Substances*. Washington: National Academy of Sciences, 1977, pp. 111–118.
12. Mello, N. K. Behavioral toxicology: A developing discipline. In: *Behavioral Pharmacology*, edited by B. Weiss and V. G. Laties. New York: Plenum Press, 1976, pp. 155–160.
13. NAS Report. Principles for evaluating chemicals in the environment. Washington: National Academy of Sciences, 1975, pp. 198–216.
14. Sidman, M. and P. B. Rosenberger. Several methods for teaching serial position sequences to monkeys. *J. exp. analysis Behav.* **10**: 467–478, 1967.
15. Spyker, J. M. Assessing the impact of low level chemicals on development: Behavioral and latent effects. In: *Behavioral Pharmacology*, edited by B. Weiss and V. G. Laties. New York: Plenum Press, 1976, pp. 161–180.
16. Thompson, D. M. Repeated acquisition as a behavioral baseline for studying drug effects. *J. Pharmac. exp. Ther.* **184**: 506–514, 1973.
17. Thompson, D. M. Repeated acquisition of response sequences: Effects of *d*-amphetamine and chlorpromazine. *Pharmac. Biochem. Behav.* **2**: 741–746, 1974.
18. Thompson, D. M. Repeated acquisition of behavioral chains under chronic drug conditions. *J. Pharmac. exp. Ther.* **188**: 700–713, 1974.
19. Thompson, D. M. Repeated acquisition of response sequences: Stimulus control and drugs. *J. exp. analysis Behav.* **23**:429–436, 1975.
20. Thompson, D. M. Repeated acquisition of behavioral chains: Effects of methylphenidate and imipramine. *Pharmac. Biochem. Behav.* **4**: 671–677, 1976.
21. Thompson, D. M. Development of tolerance to the disruptive effects of cocaine on repeated acquisition and performance of response sequences. *J. Pharmac. exp. Ther.* **203**: 294–302, 1977.
22. Thompson, D. M. Stimulus control and drug effects. In: *Contemporary Research in Behavioral Pharmacology*, edited by D. E. Blackman and D. J. Sanger. New York: Plenum Press, 1978, pp. 159–207.

Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function
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Effects of Solvents on Schedule-Controlled Behavior[1]

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COLOTLA, V. A., S. BAUTISTA, M. LORENZANA-JIMENEZ AND R. RODRIGUEZ. *Effects of solvents on schedule-controlled behavior.* NEUROBEHAV. TOXICOL. **1:** Suppl. 1, 113–118, 1979.—Operant conditioning techniques have been shown to be sensitive to the acute effects of industrial solvents. In the first experiment, five rats trained in a multiple schedule with a fixed-ratio (FR) 10 component and a differential reinforcement of low rates (DRL) 20-sec component, with a time out 60-sec between reinforcement periods, were exposed to 0.25, 0.50, 1 and 2 ml of toluene in the experimental chamber. The effects were dose-dependent, with an increase in rate in the DRL component and a decrease in FR responding. A second experiment assessing the effects of chronic exposure to thinner in the acquisition of a timing behavior in rats showed an impairment in DRL learning after 4, 8 or 16 weeks of exposure to the solvent: however, rats having a resting period did not differ from control animals. Whereas this finding suggests a reversible impairment in the acquisition of a complex behavior, further research is needed to achieve more definitive conclusions.

Operant behavior Toluene Thinner Multiple schedule Rate-dependent effects Chronic solvent inhalation Temporal discrimination Rats

RECENT interest in behavioral pharmacology and neurotoxicology has focused upon the health hazards of environmental contaminants (e.g. [16]), and on the industrial substances that are being abused for deliberate self-intoxication [13]. This concern for the effects of chemicals on behavior and bodily health and development seems to have arised from two sources of information: (a) recent reports of mass poisoning due to undetected food and water contamination [1] and of widespread abuse of solvents among youths in several countries (e.g. [4,10]): and (b) information about the pathological, long-lasting effects in central nervous system functioning due to prolonged exposure to the contaminants and industrial inhalants [11]. During the last few years, research in our laboratories has been aimed at establishing the usefulness of the operant conditioning methodology in the assessment of the behavioral effects of industrial solvents. In the first experiment we present data on the acute effects of exposure to toluene, and the second reports on the chronic effects of paint thinner inhalation in the acquisition of a temporal discrimination in laboratory animals.

EXPERIMENT 1

ACUTE EFFECTS OF TOLUENE ON OPERANT BEHAVIOR

Previous experiments [5,6] have shown the sensitivity of operant methods in the behavioral analysis of the effects of industrial solvents. It was found, for instance, that the effects of paint thinner appear to be schedule-dependent, in that responding under a fixed-ratio (FR) schedule was more sensitive than performance under a differential reinforcement of low rates (DRL) schedule, when rats trained in a Mult FR9 DRL20 schedule were exposed to different doses of the solvent [6], and that the effects of the same mixture of solvents appeared to be rate-dependent when rats trained in a fixed-interval (FI) schedule [5] were exposed to the same doses of thinner as in the other study. The present experiment extended the above findings to the main component of paint thinner, toluene.

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[1] We wish to thank Octavio Torres Cházaro for his help in the programming of the first experiment, and Jorge Rosas and Manuel González for their help in the conduct of the second experiment. Address reprint requests to: V. A. Colotla, apartado postal 69-716, México 21, D.F., México.

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METHOD

Animals

Five Wistar albino rats were used. They were approximately three months old at the beginning of the study, and were experimentally naive: the animals were kept in individual home cages and placed in a 23-hr water deprivation regime.

Apparatus

A standard BRS-Foringer operant conditioning chamber, model RG-028 was 25 cm long, 22.5 cm wide, and 20 cm in height. Both, the ceiling and the lateral walls were of transparent Plexiglas, and the anterior and posterior walls, like the 14 bars that formed the floor, were of stainless steel. In the middle of the anterior wall there was a water magazine, and 4 cm above and 4 cm to the left and right of the magazine there were two retractile levers. Only the righthand side lever was employed throughout the experiment: the left lever was kept in a retractile position.

The experimental chamber was located inside a soundproof BRS-Foringer isolation cubicle, equipped with a fan and an air extraction system. The programming and recording of events was controlled by solid state equipment. A Gerbrands cumulative recorder was also employed.

Solvent

Pure toluene ($C_6H_5CH_3$) was obtained from a commercial chemical supplier (Merck) and was kept at environmental temperature in a cool place. Toluene is the main ingredient of commercial liquid paint thinner in Mexico, and is a volatile flammable liquid, with a benzene-like odour [3].

Procedure

Each animal was trained to approach the water magazine, and shaped to press the lever for water reinforcement by the method of successive approximations. They then received a session in which every response was reinforced, and were subsequently trained to obtain reinforcements with an increasing number of responses, until stable FR10 performance was achieved. Next, a multiple FR10 DRL20 TO60 schedule was instituted, where a 60-sec time out (TO) period was followed by a 2-min period under the FR10 schedule, followed by another TO period, and an 8-min interval with the DRL20 component into effect. Thus, FR and DRL periods alternated with a TO between them. A tone present during the DRL components served as the discriminative stimulus distinguishing both reinforcement components of the multiple schedule. These sessions were 36-min long.

Three stability criteria were established before beginning with exposure to toluene: (a) an appropriate stimulus control, in that performance under each component was characteristic for that schedule: (b) a variability of no more than 10% from the average of the last three sessions in reinforcement rate (number of reinforcements per minute) under the DRL schedule: and (c) no ascending or descending trend in DRL reinforcement rate. When an animal's performance achieved the above criteria a dose of toluene was randomly selected and employed until all animals were exposed to all doses. If an animal's performance did not return to baseline level in the day following a session with the solvent, no further doses were given until the stability criteria were again satisfied. In addition, every experimental session with toluene was immediately followed by an additional session without the solvent to assess behavioral recovery. The doses employed were 0.25, 0.50, 1 and 2 ml, which given the volume of the experimental chamber (100 l), corresponded to 2.0, 4.0, 8.0 and 16.0 mg/l, or 574, 1148, 2296, and 4595 ppm, respectively.

The experimental procedure for the administration of the solvent was as follows: the dose of toluene was placed in a glass dish plate which was in turn placed underneath the grill floor of the chamber, directly below the animal. The fan and exhaust holes were covered with plastic, the animal was introduced to the chamber and the session with the multiple schedule was begun. As a control procedure, each animal was submitted to sessions in which the above procedure was repeated, but substituting the solvent by a few ml of water. These are called control sessions in the analyses of the data.

The animals had 30 min of free access to water at the end of every session, which was conducted seven days a week. Data registered included number of responses and reinforcements for each schedule component, interresponse times (IRTs) in blocks of three seconds, and response cumulative records.

RESULTS

Figure 1 shows the results obtained with one of the rats employed in the experiment. The upper record is from one of the baseline sessions and shows stable performance in the multiple schedule, with responding typical of the component schedules employed: a fast-rate FR responding and a low-rate, paced responding in the DRL schedule. The other records show the effects of the different doses of toluene, a decrease in responding in the ratio component and an increase in response rate during the DRL periods. There was also an increase in response frequency during the TO periods with the highest doses employed. The records for the other animals were similar, although in some of them FR performance was more affected than in the animal shown in this figure. There was no significant change in performance during the control sessions.

The analysis of reinforcement rate in both components is shown in Fig. 2, expressed as a percent of baseline performance. There was a decrease in reinforcement rate for both schedules under exposure to toluene, an effect that was more evident with increases in dose. FR performance was more affected than DRL responding, but the difference does not appear significant, except probably with 1 ml of toluene.

Figure 3 depicts the changes in response rate in the two schedules during the control sessions and the experimental sessions with the solvent. A differential effect is evident for the two schedules, an increase in response rate in DRL performance and a decrease in FR responding with exposure to toluene. Behavioral recovery data are still being analysed and are not reported here.

EXPERIMENT 2

CHRONIC EFFECTS OF THINNER ON OPERANT BEHAVIOR

Pathological clinical findings have been consistently reported in the literature of chronic solvent abuse [9, 11, 14]. Thus, a second experiment was conducted to evaluate the effects of chronic solvent inhalation on the acquisition of a complex behavior in laboratory rats. Since the industrial substance more widely used by solvent abusers in Mexico

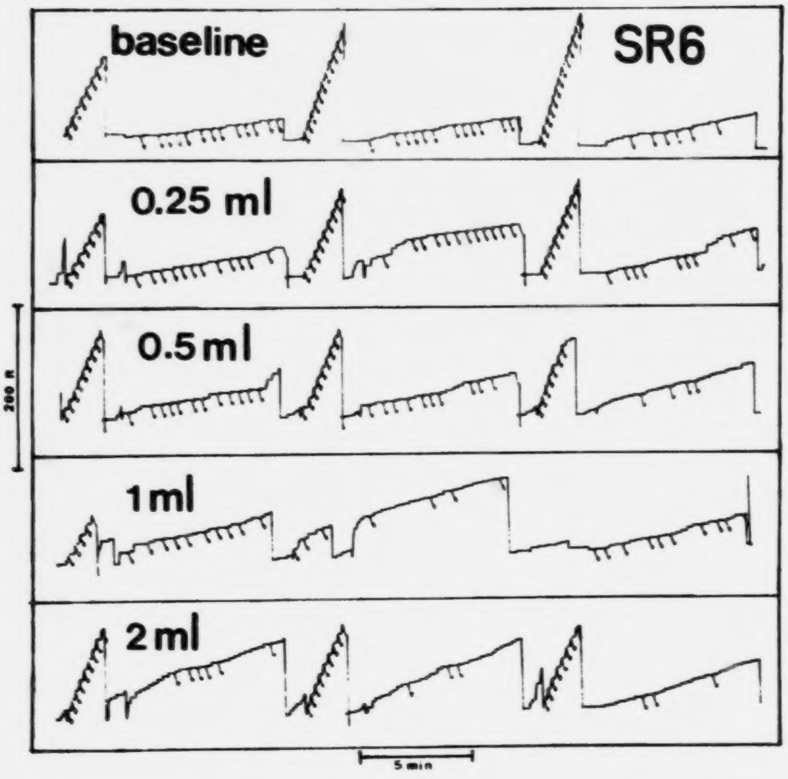


FIG. 1. Representative results obtained when rats trained in a multiple schedule were exposed to the doses of toluene indicated in the cumulative records. See text for details.

City is the commercial paint thinner easily obtained in hardware stores, the solvent employed here was the same thinner mixture (see composition below), and the behavior studied was the acquisition of a temporal discrimination as evidenced in DRL performance.

METHOD

Animals

Twenty-four Wistar albino rats were randomly assigned to one of three groups labeled A, B and C. They were all male and aged one day old at the beginning of the study. The rats remained with their mothers until weaning, which was 21 days post-partum, and were then kept in group cages with free access to food and water. Each group was further divided into two subgroups of eight animals each, an experimental (E) and a control (C) subgroup. The only restriction in the assignment to the different groups was that every experimental animal had a control counterpart from the same litter. After the chronic inhalation procedure described below all animals were transferred to individual home cages and kept in a 23-hr water deprivation regime during the operant conditioning experiment.

Apparatus

Four glass inhalation chambers, with a 3-liter volume each, were used during the inhalation treatment, maintained at a constant temperature of 25 ± 1°C. For the operant training, a BRS-Foringer operant conditioning chamber as described for Experiment 1 was employed.

Solvent

Commercial liquid paint thinner of low quality was employed. Figure 4 shows the composition of the thinner mixture by gas chromatography and the insert details some of the technical information for this analysis, and the percentage of each component of the sample.

Procedure

Inhalation treatment. All experimental animals were exposed to 131.4 mg of thinner (50,000 ppm) in the inhalation chambers during ten-min periods, twice daily, five days a week during four (Group A), eight (Group B) or sixteen (Group C) weeks. Each control rat was also placed in the inhalation chamber under the same procedure as its experimental counterpart, except that no thinner was injected into the chamber.

Operant training. At the end of the inhalation treatment for Group C (16 weeks) each subgroup of animals was further divided into two (Phase I and Phase II) to proceed with the training in the experimental operant conditioning chamber.

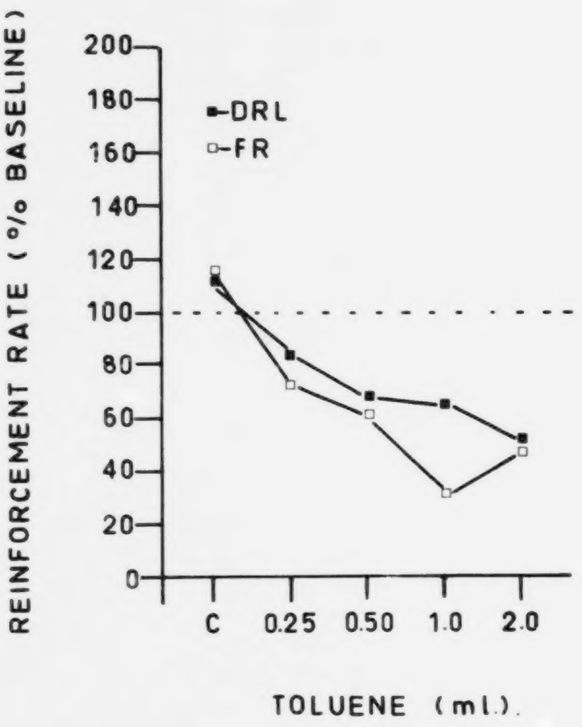


FIG. 2. Reinforcement rate as a percent of baseline in the two schedule components of the multiple schedule, under control and experimental (toluene) conditions.

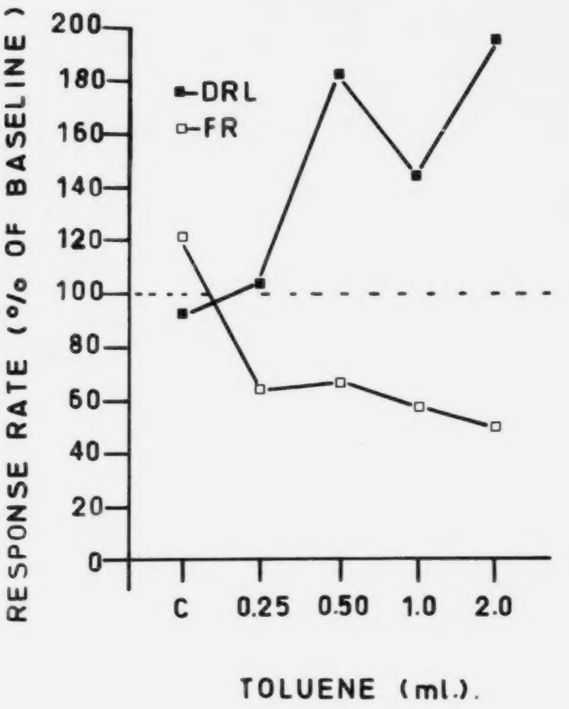


FIG. 3. Response rate as a percent of baseline in the two schedule components of the multiple schedule, under control and experimental (toluene) conditions.

Due to equipment limitations, Phase I animals were first trained and then the Phase II set of rats. The experimental procedure, however, was the same for all the animals.

Each rat was adapted to a water deprivation regime and was then subjected to the following procedure: the rat was exposed to an autoshaping situation [2], consisting of trials of a 5-sec presentation of a light above the lever followed by the free delivery of a drop of water in the magazine, every 50 sec. As expected, the pairing of light-above-the-lever and water delivery led the animal to press the lever and obtain the water reinforcement within two sessions. The rat then received two sessions in which every response was reinforced, and was then placed in a DRL20 schedule, in which a response was reinforced only if it was emitted at least 20 sec after the preceding response. Each animal received 30 sessions, each lasting 30 min, and conducted at the same time every day. Data registered were number of responses and reinforcements per session, and response cumulative records.

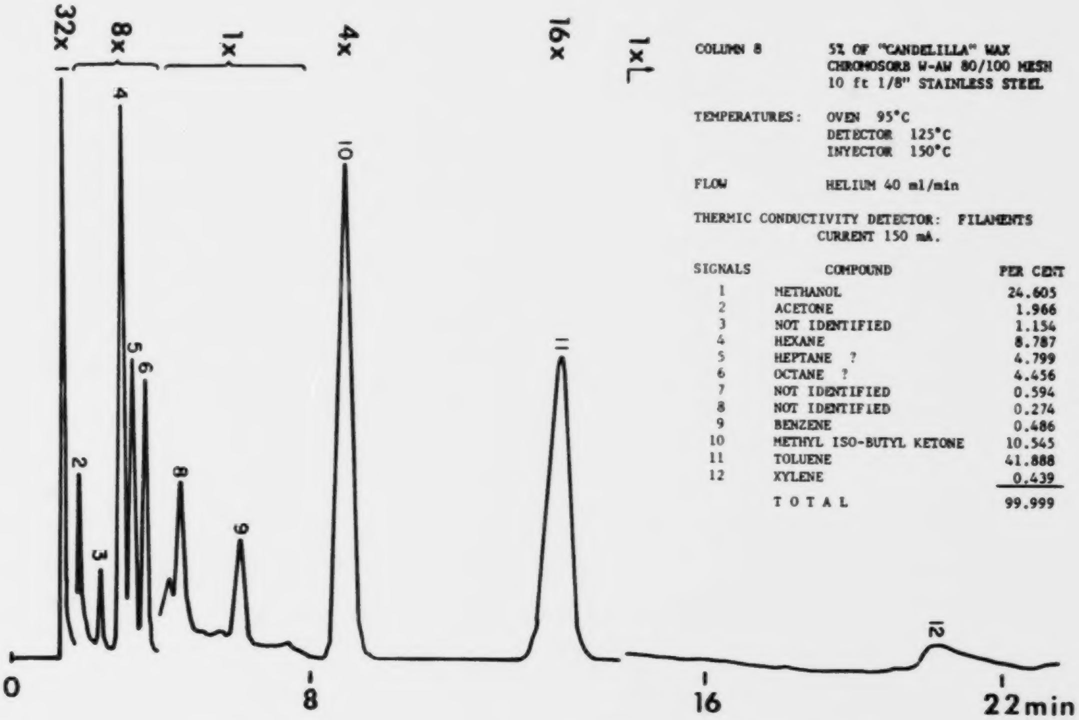
RESULTS

The main datum of interest was the percentage of reinforced responses for each subgroup of animals, taken as a measure of efficiency in DRL responding, and thus of the extent of the temporal discrimination achieved. Statistical analyses of the data showed a nonsignificant difference, $F(1,6)=0.73$, $p>0.01$, between the experimental animals in Phase I (average of 13.4 reinforced responses) and Phase II (11.6 mean reinforced responses). However, the average number of reinforced responses for the control animals was 28.0 in Phase I and 15.1 in Phase II. This difference was statistically significant, $F(1,6)=15.16$, $p<0.01$, and thus data from the two phases were not pooled. The results are shown for the two phases separately.

Figure 5 shows the main finding of this experiment. First, from the results of Phase I it is evident that the rats subjected to chronic inhalation of thinner show an impairment in the acquisition of the temporal discrimination, as compared to their control counterparts, impairment that is more marked as the inhalation treatment was extended. The rats in Phase II, however, did not show a clear differential performance with respect to the treatment of interest. Only the animals subjected to 16 weeks of thinner inhalation appeared to be more impaired than their controls.

GENERAL DISCUSSION

With respect to the first experiment, there appears to be only one report in the literature [8] which may be contrasted with the results obtained here. In that one experiment, Geller *et al.* found an increase in barpressing rate of rats trained under a variable-interval schedule when exposed to the solvent ketone, and reported a long-lasting effect in that the increased rate persisted for several days after initial exposure to the solvent. In our first experiment, however, no



COLUMN 8 5% OF "CANDELILLA" WAX
CHROMOSORB W-AW 80/100 MESH
10 ft 1/8" STAINLESS STEEL

TEMPERATURES: OVEN 95°C
DETECTOR 125°C
INYECTOR 150°C

FLOW HELIUM 40 ml/min

THERMIC CONDUCTIVITY DETECTOR: FILAMENTS
CURRENT 150 mA.

SIGNALS	COMPOUND	PER CENT
1	METHANOL	24.605
2	ACETONE	1.966
3	NOT IDENTIFIED	1.154
4	HEXANE	8.787
5	HEPTANE ?	4.799
6	OCTANE ?	4.456
7	NOT IDENTIFIED	0.594
8	NOT IDENTIFIED	0.274
9	BENZENE	0.486
10	METHYL ISO-BUTYL KETONE	10.545
11	TOLUENE	41.888
12	XYLENE	0.439
	TOTAL	99.999

FIG. 4. Gas chromatogram of the thinner mixture employed. See text for details.

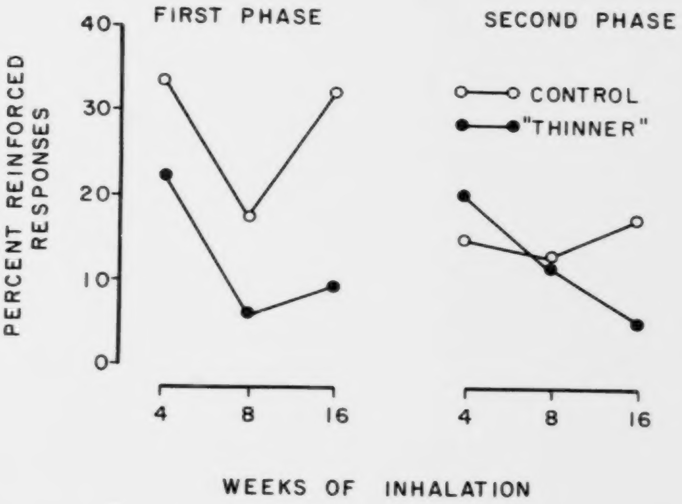


FIG. 5. Percent of reinforced responses in DRL acquisition of experimental (thinner) and control rats as a function of length of treatment (weeks of inhalation), for Phase I and Phase II animals.

persistance was found in the changes observed in response rates. Nevertheless, the main differences between the studies contrasted reside in the solvent employed and the reinforcement schedule utilized as a behavioral baseline, and thus the results should await further research for interpretation.

Furthermore, the main finding of the acute effects of the solvent on schedule-controlled behavior seems to be that there is a differential effect of toluene in the two schedules employed, which might be interpreted as a rate-dependency similar to that consistently reported for the amphetamines [7] and other behaviorally active compounds [12], a finding which would place toluene among the many other substances that interact with the behavioral rate employed as a baseline.

The second experiment was concerned with the chronic effects of exposure to commercial paint thinner on the acquisition of a temporal discrimination, as evidenced in DRL performance. The experiment followed the design employed in a study which assessed the effect of prolonged alcohol consumption in timing behavior [15], and the results suggest that persistent inhalation of thinner vapors causes an impairment in DRL acquisition, regardless of the length of treatment, when the animals are tested within a relatively short time after the inhalation procedure. However, providing a longer resting period after the exposure to the solvent results in a lack of difference between experimental and control animals, except probably those exposed for a longer time interval (i.e., 16 weeks). Whereas this finding might be interpreted as suggesting a reversibility of the brain dysfunction responsible for the impairment of the timing behavior, it should be pointed out that the control rats in Phase II had a poor performance as compared to Phase I controls, a fact that should be examined more closely in further experimentation. It appears then, that chronic exposure to thinner may cause an impairment in the acquisition of a complex behavior in laboratory animals.

REFERENCES

1. Bakir, F., S. F. Damluji, L. Arnin-Zeki, M. Murtadha, A. Khalidi, S. Tikriti, H. I. Dhakir, T. W. Clarkson, F. C. Smith and R. A. Doherty. Methylmercury poisoning in Iraq: An inter-university report. *Science* **181:** 230–233, 1973.
2. Brown, P. L. and H. M. Jenkins. Autoshaping of the pigeon's keypeck. *J. exp. analysis Behav.* **11:** 1–8, 1968.
3. Bruckner, J. V. and R. G. Peterson. Review of the aliphatic and aromatic hydrocarbons. In: *Review of Inhalants: Euphoria to Dysfunction.* NIDA Research Monograph 15, edited by C. W. Sharp and M. L. Brehm. Rockville, Maryland: National Institute of Drug Abuse, 1977, pp. 124–163.
4. Carroll, E. Notes in the epidemiology of inhalants. In: *Review of Inhalants: Euphoria to Dysfunction.* NIDA Research Monograph 15, edited by C. W. Sharp and M. L. Brehm. Rockville, Maryland: National Institute of Drug Abuse, 1977, pp. 14–17.
5. Colotla, V. A., B. E. Z. Jacobo and M. M. G. Moctezuma. Efectos agudos del "thinner" en la ejecución de ratas en un programa de intervalo fijo. *Rev. Mex. Análisis Cond.* **4:** 1978, in press.
6. Colotla, V. A., M. Lorenzana-Jiménez, J. Echavarria Luna and R. Rodriguez. Evaluación de los efectos conductuales del tiner con la metodología operante. *Cuad. Cient. CEMESAM* **8:** 203–220, 1978.
7. Dews, P. B. and G. R. Wenger. Rate-dependency of the behavioral effects of amphetamines. In: *Advances in Behavioral Pharmacology*, edited by T. Thompson and P. B. Dews. New York: Academic Press, 1977, pp. 167–227.
8. Geller, I., J. R. Rowlands and H. L. Kaplan. Efectos de las cetonas en la conducta operante en animales de laboratorio. In: *Inhalación Voluntaria de Disolventes Industriales*, edited by C. M. Contreras Pérez. México: Editorial Trillas, 1977, pp. 125–138.
9. Grabski, D. A. Toluene sniffing producing cerebellar degeneration. *Am. J. Psychiat.* **118:** 461–462, 1961.
10. Natera, G. Estudio sobre la incidencia del consumo de disolventes volátiles, en 27 centros de la República Mexicana. In: *Inhalación Voluntaria de Disolventes Industriales*, edited by C. M. Contreras Pérez. México: Editorial Trillas, 1977, pp. 329–351.
11. Prockop, L. and D. Couri. Nervous system damage from mixed organic solvents. In: *Review of Inhalants: Euphoria to Dysfunction.* NIDA Research Monograph 15, edited by C. W. Sharp and M. L. Brehm. Rockville, Maryland: National Institute of Drug Abuse, 1977, pp. 185–198.
12. Sanger, D. J. and D. E. Blackman. Rate-dependent effects of drugs: A review of the literature. *Pharmac. Biochem. Behav.* **4:** 73–83, 1976.
13. Sharp, C. W. and M. L. Brehm (eds.) *Review of Inhalants: Euphoria to Dysfunction.* NIDA Research Monograph 15. Rockville, Maryland: National Institute on Drug Abuse, 1977.
14. Tsushima, W. T. and W. S. Towne. Effects of paint sniffing on neuropsychological test performance. *J. abnorm. Psychol.* **86:** 402–407, 1977.
15. Walker, D. W. and G. Freund. Impairment of timing behavior after prolonged alcohol consumption in rats. *Science* **182:** 597–599, 1973.
16. Weiss, B. and V. G. Laties. *Behavioral Toxicology.* New York: Plenum Press, 1975.

Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function
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Testing for Behavioral Effects of Agents

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DEWS, P. B. AND G. R. WENGER. *Testing for behavioral effects of agents*. NEUROBEHAV. TOXICOL. **1:** Suppl. 1, 119–127, 1979.—In the present state of science no morphological or chemical changes may be detectable at a time when behavior is profoundly disturbed, as in schizophrenia. Until we are reassured to the contrary, we must assume that exogenetic intoxication can produce changes detectable only as behavioral changes. Therefore behavioral toxicology must be studied. In contrast to toxic manifestations such as lethality or carcinogenicity, which tend to be unequivocal and irreversible, behavioral changes are like physiological changes in that they are quantitative, changing in time, and relate to variables with a considerable range of normal variability. An experiment on behavioral teratology in mice is described and the results used to illustrate the limits of the possible in behavioral toxicology. From reported and observed variability it is surmised that changes that occur in as many as 1 per 100 of the population or average as large as a 10% decrement will still be too small to be detected by direct experiment. Such risks are frequently unacceptable. Reasons are given for hoping that epidemiological studies may be able to supplement experimental toxicological studies to provide a better assessment of risk of small impairments or rare susceptibility.

Mice Caffeine Atropine Behavioral toxicity Behavioral teratology Safety-testing Mult FR 30 FI 600 sec Schedule-controlled responding Spontaneous motor activity

THERE are many reasons for studying the behavioral effects of toxic influences but one reason is fundamental: in the present state of science no morphological or chemical changes may be detectable at a time when behavior is profoundly disturbed. The most dramatic example is acute schizophrenia when almost any psychological or psychiatric test will show gross abnormality while there are no detectable morphological changes and all chemical deviations found to date are variable, not diagnostic, and can be related to changes in dietary and other life patterns consequent on schizophrenia. It is not necessary to assume that schizophrenia is an intoxication for the importance of behavioral studies to be emphasized by the example. If the undiscovered influence in schizophrenia can cause such profound changes in behavior then it must be assumed that exogenetic intoxication can do the same, until we are reassured to the contrary. There is insufficient evidence for us to be generally reassured at the present time, although it appears likely that profound behavioral disturbances not accompanied or even preceded by other easily detectable evidences of intoxication occurs with very few agents. The analogy to schizophrenia may be taken one step further. Almost any behavioral test will detect abnormality in a schizophrenic. Perhaps at the present stage of behavioral toxicology we should concentrate on tests that are quick, efficient and informative and not be too concerned about finding tests whose results have plausible theoretical interpretations.

Other reasons for studying behavioral toxicology are as follows. Behavioral tests are non-destructive so a subject can be studied repeatedly. Thus the development of and perhaps recovery from toxicity can be followed in a manner not possible for histological and many chemical assays. There is a widespread expectation that behavioral changes may be an early manifestation of toxicity and that therefore behavioral tests will provide very sensitive indices of toxicity. Many people have gone so far as to suggest that the reason for studying behavioral toxicity is that it might provide the most sensitive tests, and that if behavioral tests are not generally the most sensitive then behavioral toxicity is not worth studying. As indicated above, the latter premise is unacceptable. Whether behavioral tests are usually more sensitive is a question to be answered by experiments. There is relatively little in the way of systematic comparisons in the Western literature. There is a formidable body of opinion in the USSR that for a great many agents behavioral tests are the most sensitive indications of toxicity, and maximum allowable concentrations in the USSR based on behavioral tests are much lower than in the US where they are usually based on other criteria [5]. Another reason for studying behavioral toxicity is the hope that the effects of poisons will help the analysis of behavioral phenomena in a way similar to the large role that poisons played in the analysis of physiological phenomena, from Claude Bernard and his curare to tetrodotoxin, bungarotoxin and actinomycin in our own time. Finally, some people study behavioral toxicity simultaneously with neurological and neurochemical toxicities in the hope of gaining insight into the normal relations among behavioral, neurological and neurochemical phenomena.

The discussion of "test methods for the definition of effects of toxic substances on behavior" will be approached through the description of a simple pilot experiment. The pilot experiment was actually an experiment in behavioral teratology, but serves to illustrate problems general to behavioral toxicology and indeed to toxicology and safety-testing in general. Teratological effects have the advantage for behavioral studies that they may be permanent and relatively stable.

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When pregnant subjects are exposed to a teratogen, typically only occasional offspring are terata, the remaining members of the litters being indistinguishable from normal. The frequency of terata increases with increasing exposure to the teratogen at the critical periods during pregnancy. Teratology has been primarily a morphological science, and terata have been recognized by their anatomical deviances. (Indeed, Webster's dictionary defines teratology as concerned exclusively with deviations from normal *structure*. The legitimacy of *behavioral* teratology as a designation would appear to derive from equating terata with monsters: monsters may be monstrous by reasons of their behavior, again according to Webster. It would have been better to invent another term to cover the behavioral consequences of antenatal influences but it is too late now.) It is only in recent years that there has been much interest in the possibility that permanently deviant behavior may result from exposure to particular chemical substances during development, in the absence of currently recognizable morphological or physiological deviance. It is recognized that permanent behavioral deviance must be the result of permanent physiological changes; it must also be recognized, however, that the physiological changes may not be indicated by any available technique other than behavioral assessment.

That permanent changes in behavior may result from in utero exposure to teratogens is undoubted. What is much less clear, however, is whether permanent changes in what the organizers of the workshop have chosen to call cognitive behavior may result from exposures that do not have other, more easily detectable, physical effects such as interference with pregnancy itself, reduced survival of litters, lower weight gain in offspring, and increased incidence of familiar morphological terata or grossly obvious behavioral incompetences. Most, if not all, examples of behavioral teratology in the literature have employed exposures that have had clear effects detectable by conventional teratologic techniques or by such minor modifications as letting the offspring grow up and looking at them and weighing them.

A fundamental consideration in behavioral teratology does not seem to have been addressed definitively. If behavioral terata occur as do anatomical terata, then most prenatally exposed offspring should be normal with only occasional individuals showing gross behavioral changes that should be fairly easily detected by simple general tests even if the changes are specific. To detect such teratogenesis simple and speedy tests should be used on a prolific species so that as many subjects as possible can be examined. Alternatively it may be suggested that during central nervous system maturation so many different pathways and connections must develop in exactly the right timing and sequence that most subjects exposed to a sufficient noxious influence would have some one or other process more or less perverted. Higher behavioral activities require a great deal of sequential processing in the CNS so that there is a high likelihood that if deviances exist they will be encountered in the processing. The results might be any one or more of a variety of deviances, perhaps slight or subtle, but occurring in most exposed subjects. Such impairments are most likely to be detected by intensive study of a few subjects. The alternative suggestions thus point to different strategies for safety testing. Our pilot experiment addressed, but did not solve, this issue.

PILOT EXPERIMENT

The effects of atropine or caffeine throughout the period of development of the CNS were studied in mice. Atropine was selected because its high activity in antagonizing muscarinic effects of acetylcholine suggested it might interfere with cholinergic function in the developing nervous system, with permanent sequelae. Caffeine was chosen as an agent of wide distribution that can have behavioral effects and in massive doses is conventionally teratogenic [7]. It was of interest to see whether smaller doses produce behavioral teratology.

METHOD

Female mice in cages with males were subjected to a 12-hr light/12-hr dark diurnal cycle and examined each morning for vaginal plugs. When a plug was observed, the mouse was randomly assigned to one of eight groups and housed individually (Table 1). The mice were exposed to the various solutions or water as sole drinking fluid throughout pregnancy. Litters were culled to 6 on the day of birth, and exposure to the solutions continued.

Preliminary experiments suggested that mice restricted to 0.3 ml/g/day of drinking fluid would essentially always drink all the fluid within 24 hr, so that dosages of agents could be known, and that this fluid intake would support normal growth. Accordingly, all groups of mice were fluid restricted except one control group given unlimited water. All restricted mice essentially always drank all their fluid, and maintained normal growth rates except, perhaps, for some slight slow-down in growth of pups just before weaning. At 21 days, the litters were separated from the dam, and separated by sex, but they continued to receive only the solutions as drinking water until 45 days of age. All litters were then given unlimited water for the rest of the study. Testing was started at 60 days. No attempt was made to conduct simultaneously classical teratological assessments (disssections and histology). Three behavioral tests were used. Test 1 was a simple assessment of spontaneous locomotor activity (SMA). A transparent plastic mouse cage was traversed by a light beam. A single mouse was put in the cage and the number of times the light beam was broken during a period of one half hour was counted. Test 2 was a motor activity test rather similar to the first but with an additional feature. The mouse was studied in a circular cage with 3 light beams and 3 photocells (Fig. 1). The beams (1, 2 and 3) made it possible to know when the mouse was continuing round and round in the same direction rather than backing and hauling. The total number of times any light beam was broken and the number of times the mouse broke the beams in the sequence 1 then 2 then 3 (rotation) were counted. The same ten mice of each sex from each treatment group were studied in Tests 1 and 2 for 3 consecutive days starting shortly after the 60th day of age. Test 3 was an assessment of schedule-controlled responding. Mice broke repeatedly a beam of light falling onto a photocell by moving their noses in and out of the beam and received milk under a mult FR30 FI 600 sec schedule [9]. One male and one female mouse from each of 3 litters in each of the 8 groups was trained. Each mouse was studied daily 5 days/week for 5–6 weeks (which is why only 3 males and 3 females per group could be studied). The mice were exposed

TABLE 1

Regimen	Designation	Concentration	Volume
ad lib water	ad lib	0	ad lib
restricted water	R-H_2O	0	0.3 ml/g/day
10 mg/kg/day caffeine	10 CAF	0.033 mg/ml	0.3 ml/g/day
30 mg/kg/day caffeine	30 CAF	0.10 mg/ml	0.3 ml/g/day
100 mg/kg/day caffeine	100 CAF	0.33 mg/ml	0.3 ml/g/day
0.3 mg/kg/day atropine	0.3 AT	0.001 mg/ml	0.3 ml/g/day
3.0 mg/kg/day atropine	3 AT	0.01 mg/ml	0.3 ml/g/day
30 mg/kg/day atropine	30 AT	0.10 mg/ml	0.3 ml/g/day

to a standard training sequence, much as previously described [9]. The final schedule was continued for 13 sessions, and responses in last 3 sessions averaged for tabulation. The number of times during training that a session was not completed in a nominal time (30 min) was also noted.

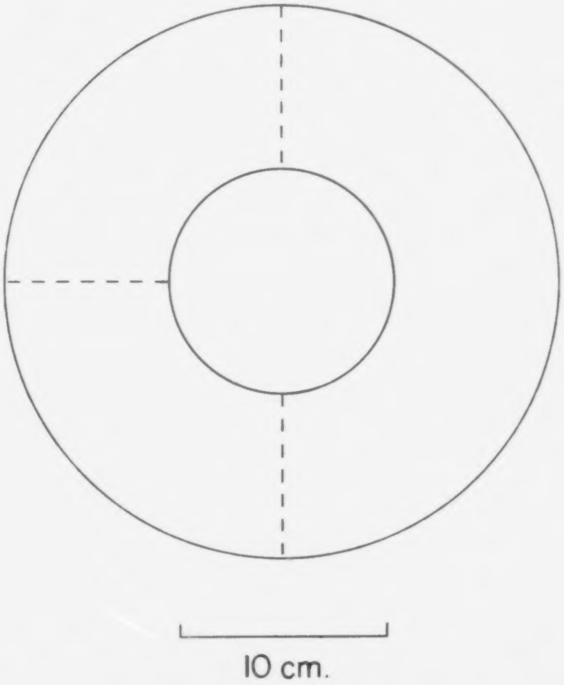

FIG. 1. Dotted lines show beams of light crossing circular track in which mouse is placed for 30 min.

Comments

The simple assessments of spontaneous motor activity, Tests 1 and 2, permitted a reasonably large number of mice to be studied. Test 3, mult FRFI, represents a higher behavioral activity, suitable for intensive appraisal of a few mice. The schedule is well established as sensitive and productive of valuable information in behavioral pharmacology [4] and has already been used in behavioral toxicology [6].

RESULTS

Test 1

The average total counts per mouse in three sessions on three consecutive days for the different treatment groups are shown in Table 2. All the counts were between 540 and 647 with SE between 32 and 87 save for one group, the females that had been raised on 100 mg/kg/day caffeine. This group had a mean of 838 and SE of 273. The high SE suggests that the high count may be due to a few high values. In fact it was due to a single mouse that had a count of 2723. The next highest count among the remaining 159 mice tested was 1362 and only two other mice had as many as 1000. None of the mice in the other treatment groups differed from one another or from those receiving free water: notably, the large dose of 30 mg/kg/day of caffeine and all doses of atropine were without recognizable effects.

Test 2

The total number of breaks of the three light beams are shown in Table 3. The results were unremarkable except for the high variance in the females of the 100 mg/kg/day caffeine group, which turned out to be due to the same mouse as the high counts in Test 1. The numbers of complete clockwise circumnavigations of the cage (rotations) distinguishes the female of the 100 mg/kg/day group even more clearly. The high variance of this group is seen in Table 4. The single abnormal mouse in relation to the other 159 mice studied is identified in the frequency-distribution of Fig. 2: the score of

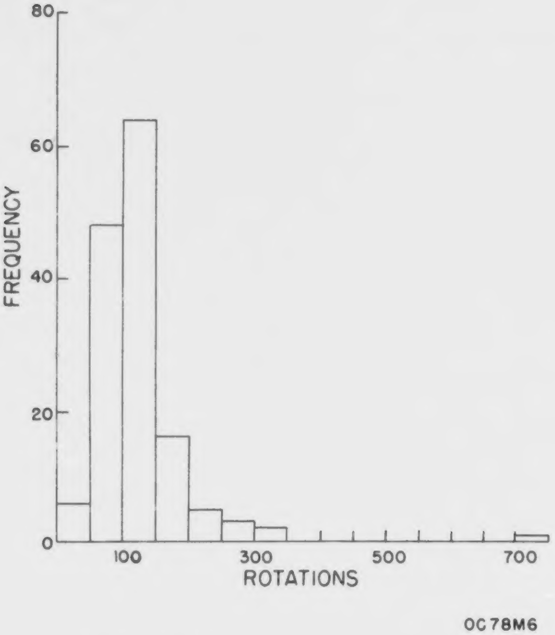

FIG. 2. Frequency-distribution of different numbers of rotations in three sessions of 160 mice.

TABLE 2

ACTIVITY 1/90 MIN

	M		F		M&F
	m	SE	m	SE	m
ad lib	604	32	647	87	626
RH_2O	544	47	581	49	562
10 CAF	602	56	608	36	605
30 CAF	502	56	593	50	548
100 CAF	557	73	838	273	698
0.3 AT	565	36	532	37	548
3 AT	540	51	606	34	573
30 AT	588	66	509	64	548

TABLE 3

ACTIVITY 2/90 MIN

	M		F		M&F
	m	SE	m	SE	m
ad lib	3913	253	4252	414	4082
RH_2O	3540	161	4954	463	4247
10 CAF	3472	129	4114	268	3793
30 CAF	2987	271	4308	296	3648
100 CAF	3297	143	3987	591	3642
0.3 AT	4046	279	4202	268	4124
3 AT	3485	248	3989	270	3737
30 AT	3878	242	3828	242	3853

TABLE 4

ACTIVITY: ROTATIONS/90 MIN

	M		F		M&F
	m	SE	m	SE	m
ad lib	164	22	130	22	147
RH_2O	108	9	147	44	139
10 CAF	116	7	103	11	110
30 CAF	101	15	116	11	109
100 CAF	94	11	174	79	134
0.3 AT	117	14	96	9	106
3 AT	137	21	113	16	126
30 AT	137	25	128	12	133

this one mouse is 3 SD's to the right of the mean. High activity can arise from a variety of abnormalities unrelated to caffeine so, obviously, no conclusion can be drawn from a single mouse.

Test 3

All the mice trained under mult FR30 FI300 sec gave similar performances without regard to early treatment, with again, a single exception. No specific change in any aspect of multi FRFI responding in most mice exposed to early treatment with caffeine or atropine has been identified.

Table 5 summarizes information of FR. The mean rate of responding was about 1 response per second, females a shade lower than males, but not significantly so. Rates for 100 mg/kg/day caffeine group were low but not lowest for either males or females although for the average of both they were, in fact, the lowest. The main reason for this turned out again to be a single mouse that had a very low FR rate. After responding normally for some sessions the FR rate declined to very low levels in this mouse. Meanwhile, FI responding remained substantially normal.

Spontaneous loss of FR pattern of responding is known to occur not infrequently. It has been seen many times in a variety of species down the years when no caffeine was involved. FR responding has a positive feedback feature. The faster the subject responds, the sooner the food is delivered; and it has been suggested that this feature favors faster responding, which in turn brings food still closer which further favors still faster responding and so on until the subject approaches its physiological limit of rate of responding. Indeed, the fastest rates of responding that have ever been recorded have been under these FR type schedules. One of the lines of evidence for the positive feedback feature is that it also seems to work in the opposite direction. If rate of responding is slowed, food comes slower which favors slower responding and so on until responding becomes desultory. As an isolated finding, little significance can be attached to a not uncommon phenomenon occurring in one mouse.

Table 6 shows data for FI. There were no consistent convincing differences between mice of any of the treatment

TABLE 5

FR (R/SEC)

	M		F		M&F
	m	SE	m	SE	m
ad lib	1.01	0.46	0.63	0.08	0.82
RH_2O	1.23	0.44	0.87	0.21	1.05
10 CAF	1.72	0.48	1.45	0.54	1.58
30 CAF	1.00	0.12	0.84	0.13	0.92
100 CAF	0.77	0.34	0.56	0.36	0.66
0.3 AT	0.73	0.11	0.87	0.11	0.80
3 AT	1.20	0.18	0.55	0.43	0.88
30 AT	1.59	0.18	0.86	0.24	1.22

TABLE 6

FI (R/SEC)

	M		F		M&F
	m	SE	m	SE	m
ad lib	0.39	0.12	0.29	0.10	0.34
RH_2O	0.34	0.02	0.33	0.12	0.34
10 CAF	0.44	0.08	0.52	0.07	0.48
30 CAF	0.35	0.15	0.31	0.03	0.33
100 CAF	0.28	0.11	0.47	0.23	0.38
0.3 AT	0.27	0.04	0.42	0.12	0.34
3 AT	0.32	0.07	0.17	0.08	0.24
30 AT	0.66	0.09	0.32	0.09	0.40

groups under FI. The variability was again highest in the females that had received 100 mg/kg/day caffine.

Training

In the training sessions for mult FRFI, the numbers of times there was a failure to complete a session in 30 min (see Method) in the 6 mice in each of the various groups was as follows: ad lib, 4: restricted water, 7: 10 mg/kg/day caffeine, 7: 30 mg/kg/day caffeine, 4: 100 mg/kg/day caffeine, 10: 0.3 mg/kg/day atropine, 1: 3 mg/kg/day atropine, 1: 30 mg/kg/day atropine, 6. Once again it is the 100 mg/kg/day caffeine group that gives the extreme value although again, not statistically significantly so, and dose-effect relations are not apparent, casting serious doubt on the biological significance.

Comments

Three independent suggestions of behavioral abnormality in the 100 mg/kg/day caffeine treated offspring were found. None of them is, in itself, at all convincing, but the coincidence of the 3 extreme values occurring in the same one of the 8 groups is not easily dismissed $((1/8)^3=0.002)$. Even more important is that other workers have found behavioral teratology at these sorts of levels of exposure in rats (Sobotka, private communication).

If it is accepted that the tests showed the occurrence of real behavioral teratology, what light is thrown on the issue posed at the beginning: is behavioral teratology a phenomenon of sporadic occurrence among exposed offspring or is it a matter of graded impairment of many? At face value, the evidence certainly suggests sporadic occurrence of big deviances with the large dose of caffeine. The evidence is compatible with the nature of the deviance being of very different type from pup to pup, just as a single teratogen may produce very different types of morphological terata. No evidence of impairment of most of the pups raised on 100 mg/kg/day was detected. But the fact is inescapable that small changes could not have been established. For example, the low rate of responding under FI of the females raised on 3 mg/kg/day atropine was not statistically significant. In all the tests, coefficients of variation were in the vicinity of 0.3, and some of this variation is non-random. The implications of high variability with a component not due to sampling error has been discussed previously [2] and will be elaborated later.

TABLE 7

PREGNANCIES

	Plugs	Pregs	Non-preg Plug
ad lib	6	6	0
RH_2O	8	6	0.25
10 CAF	21	17	0.19
30 CAF	8	8	0
100 CAF	24	16	0.33
0.3 AT	5	4	0.20
3 AT	5	5	0
30 AT	5	5	0

Other Effects

Although the pilot study was concerned with behavioral toxicology, some routine biological information was collected.

(1) As indicated in Method, mice were allocated at random to the various groups after diagnosis of a vaginal plug. The incidence of pregnancies is shown in Table 7. The incidence was lowest in the 100 mg/kg/day caffeine group. The other groups showing a less than 100% pregnancy rate were the restricted water and the low doses of both of the agents. The apparent paradox may be related to the relative consumption of the various solutions. With water and the low concentrations of agents, all fluid tended to be drunk in a limited period following its presentation each day, leaving the subjects without fluid for a substantial fraction of each day, while with the higher concentrations, fluid was taken more slowly through most of the 24 hours. It may be that periods without drinking fluid are deleterious to pregnancy.

(2) Mean litter size was not affected by caffeine (Table 8) in agreement with other reports [7].

(3) Two pups out of 30 on 100 mg/kg/day caffeine failed to survive to 21 days while only 1 of the 180 in all the other groups died in the first 21 days. This difference has less than a 1 in 10 chance of occurring as a sampling error.

(4) At 60 days, there was no difference in mean body weights between the groups (Table 9).

TABLE 8

MEAN LITTER SIZE

	m	SD
ad lib	8.8	2.4
RH_2O	11.6	1.0
10 CAF	10.1	2.1
30 CAF	9.8	2.3
100 CAF	9.6	2.6
0.3 AT	10.0	1.6
3 AT	7.8	2.8
30 AT	11.4	0.9

TABLE 9

WEIGHTS AT 60 DAYS (g)

	M	F	M&F
ad lib	27.6	23.1	25.4
RH_2O	25.3	21.8	23.6
10 CAF	29.6	23.9	26.8
30 CAF	27.7	20.4	24.0
100 CAF	30.1	22.1	26.2
0.3 AT	28.4	21.8	25.1
3 AT	28.1	21.2	24.6
30 AT	27.4	22.3	24.8

Comments

Caffeine at 100 mg/kg/day slightly reduced the viability of fetuses and infants. No behavioral effects were detected at exposures of less than 100 mg/kg/day. Thus it was only at an exposure that reduced viability that behavioral changes were detected. Is this assurance that caffeine is not a behavioral teratogen at doses less than 100 mg/kg/day? In itself it is very little assurance. If the incidence of detectable behavioral terata were as high as 1% there is only a 20% chance that an instance would occur in a sample of 20 mice. (With incidence of 1% of terata there is a probability of 0.99 that a subject will be normal. The probability of all of a sample of 20 being normal is $(0.99)^{20}=0.818$, so there is only about a 20% chance of one or more abnormalities occurring in the sample of 20.) To have a less than 1% chance of missing an incidence as horrendously high as 1% would require over 450 subjects. (For a 99% chance of having at least one abnormality in the sample, the sample size n would have to be such that. . .

$$\ldots \qquad (0.99)^n = 0.01$$

$$\ldots \text{ or } n \log 0.99 = \log 0.01$$

$$n = \frac{\log 0.01}{\log 0.99} = \frac{-2.00}{-0.00436}$$

$$= 458$$

For similar assurance of detecting an abnormality with the still unacceptably high incidence of 1 in 100,000 would require nearly half a million subjects. (If the incidence is 1 in 100,000 the corresponding n is given by. . .

$$n = \frac{\log 0.01}{\log 0.99999} = 460,515$$

Further, because of the high variability encountered in assessment, even an abnormality that occurred in most subjects would have to be large to be detected. With a coefficient of variation of 0.3, a change of 20% would be necessary to attain statistical significance on 20 subjects. (With a coefficient of variation of 0.3, a change averaging 20% in 20 subjects would give a t value of $0.2 \div (0.3/\sqrt{20}) = 2.98$. The $p<0.01$ value of t for 19 d.f. is 2.86). As some of the variance is not due to random sampling errors [2,3], it is likely that less than, say, a 10% change could not be detected, no matter how many subjects were studied. A decline of 10% in any productive behavioral function is quite unacceptable. Finally, only the possibility of detecting change has been considered. Changes in themselves do not prove serious toxicity as they may be transient or even sought after, as with therapeutic agents, so more testing would be indicated when changes were detected.

Some of these limitations arise from the exigencies of behavioral teratology where after long and laborious preparation, each subject can only contribute one datum. In behavioral pharmacology in which each subject can function as its own control in split sessions on each day, coefficients of variation of mice under FR schedules of less than 0.05 have been attained. But there will always be variability in test measures, not all of it due to sampling errors, and infrequent occurrences or small changes will remain undetectable whether the changes are in behavioral functions or in physiological functions that require time to measure. Consider, for example, how hard it would be to detect a long term decline of 10% in cardiac output, ventilatory capacity or red cell count and to attribute the change to a specific agent.

It should be remarked parenthetically that assurance of the safety of caffeine does not depend on studies such as the one described but rather on the vast amount of scientific work, laboratory as well as epidemiological, that has been performed on caffeine attesting to its harmlessness as used by sane human beings.

But what about new agents that have been studied little or not at all? What follows addresses the problems faced by the Office of Toxic Substances (O.T.S.) in meeting their mandated concern for behavioral testing. Many of the general points apply to testing for other types of toxicity and for other classes of agents, such as those regulated by the Food and Drug Administration.

First, safety-testing, in seeking to prove the Null Hypothesis that an agent causes no harmful effects, starts from a logically unsound position, and the sooner we educate the people and the Congress that they must accept this unpalatable truth, the better it will be for consumers, producers, and testers. Risks can be assessed but it is fundamental that to assess a risk one must have an effect, and further, for quantitative risk assessment not only an effect but a dose-dependent effect is needed so that the slope of the dose-dependent curve can be estimated. Such information may be inadequate for realistic assessment of risks of very low exposures such as human populations might receive, but it is surely necessary as a starting point.

Second, while serious toxic effects in 1 in 100,000 or even 1 in 1,000,000 of exposed people or toxic declines in functions of even a few percent are unacceptable, it is hard in blind testing to establish in animals incidences as high as 1 in 100 or declines as great as 10%. By "blind" testing is meant testing when there is no indication of what to look for in the way of effects and therefore the attempt is made to detect any and every deviance that might occur. The problem of extrapolation to low incidence has been discussed most in regard to carcinogens: paradoxically, not because it is more formidable with respect to carcinogens but rather because it appears to be so much easier that some have been deluded into believing it possible. Cancer is able to be diagnosed with greater certainty than almost anything else, except death, and it persists for confirmation and can be documented permanently on a microscope slide with almost zero variance. Compare that with a behavioral or physiological assessment. Even a grossly deviant behavioral or physiological finding in a single mouse carries little conviction of significance. An intercurrent infection unrelated to the agent can cause such deviance. What we need for conviction are consistent findings in a number of mice systematically related to load of agent in the form of a dose-effect relationship. The exploration of such a relationship even in the 10^{-2} range involves impossibly large numbers of subjects. The same applies to behavioral changes less than about 10% (10^{-1}) even if they occur in most subjects. If a dose-effect curve is sigmoid, and, properly plotted, essentially all of them are, then in seeking a minimum effective dose we are working with a part of the curve where the slope approaches zero as an asymptote, where a large difference in dose makes almost no difference to the effect and so, conversely, a small error in measurement of the effect makes an enormous difference to the estimate of minimum effective dose. If we are attempting to determine the dose that kills less than 1 per 1000 mice and one love-lorn mouse dies of a broken heart or because an animal handler dropped a cage lid on him or her yesterday, then that little mishap vitiates results on thousands of mice, or worse, leads us to a wildly wrong answer.

The general approach to safety-testing seems to be to try to administer an agent to a reasonably large number of rats for as long as reasonably possible, 18 months to 2 years, or even multigeneration, in as high a dosage as feasible, the maximum that is tolerated without debility (M.T.D.). A reasonably large number is frequently hundreds. The tests are geared to measurements of body weight, hematology (because blood is easily accessible) and to organ weights and histology post mortem. It is pathology oriented rather than pharmacology oriented.

Safety-testing has another feature. In safety-testing, all the immediate pressures are not to find anything. The manufacturer of an agent wants the testing to show that it is safe, that is, for the studies to find nothing. Positive findings can really be quite traumatic to the discoverer of them. As most people most of the time are successfully finding nothing, the unfortunate who makes a discovery is likely to have his company besmirched on evening TV and in the morning paper. Regulatory agencies also have some reason to prefer negative findings. Positive findings generate all sorts of political pressures and require hard decisions. Life is simpler and pleasanter if nothing is found. Regulatory agencies try to protect themselves by insisting on MTD's and GLP's but both bring their own train of problems. The regulatory agencies are starting to build their own in-house research groups, who are already invaluable as eagerly-looking for positive effects, but the groups will remain small in relation to the size of the tasks for the foreseeable future. Very few academic scientists work in the field. Consumer activists do not have the money for testing and in any case they are sure they know the answer: that nothing is safe. No one is being accused of dishonesty nor even lack of dedication. Indeed, most negative findings reflect that there are no effects of the agents as tested and assessed so the results are replicable and correct as presented. Doing test after test with negative results is not, however, the best way to maintain alert interest to recognize the occasional test when something unexpected happens. Further, the design, conduct and interpretation of a test may be subtly altered when the best result is no result, especially when we are dealing with low risks. Schneiderman, Mantel and Brown [8] say: ". . . laboratory errors, considered as noise, could tend to overwhelm any signal present in the data, making it still more difficult to differentiate between treated and control animals. The statistical remedy is to enlarge the experiment by yet another large factor increasing the risk of further blunders that could wipe out the gains of the increased experiment size. In this kind of experiment if one wanted to show 'safety' of a material, quite possibly pressures would exist to do a poor job. The more errors made, the less likely it is to show a difference between treated and controls." Conscientious clinicians have accepted the necessity for so-called "double-blind" designs in the assessment of therapeutic procedures. I am not advocating "double-blind" procedures for safety testing except perhaps in a few special cases. The example of clinicians is used to indicate that one does not have to accept that one's honor is impugned to recognize the possibility of bias.

Routine testing is inevitably directed toward preventing previous disasters from happening again. Nothing could be more destructive of imaginative approaches to devising means of anticipating and preventing future disasters than massive routine testing programs with preoccupation with trivial procedural details imposed by GLP leaving no time, inclination, or incentive to think. Routine blind subacute and chronic toxicity testing of the limitless series of agents to which we are exposed cannot provide the information needed and is enormously expensive. It should be a last resort.

The law of diminishing returns seem to operate with unusual force in toxicology. One can get a rough estimate of LD50 with as few as a dozen or so mice (an assessment that would probably have been sufficient to prevent the Elixir or Sulfanilamide tragedy), and a similar number could indicate where the dose-effect curve lay for a behavioral effect such as change in spontaneous motor activity (SMA). A very large number of agents could be studied in these two simple assays. To add a clinical assessment of the mice would increase the cost manyfold. It remains to be shown that a clinical examination can provide reliable evidence of abnormality in mice that show normal SMA, or that a far more elaborate screen provides more than a meagre increase in information at a disproportionately large increase in demand on resources. Evidence that an agent accumulates and therefore kills when given subacutely in a fraction of the acute LD50 can be obtained on a very few mice. Again to get much more information involves an enormous increase in investment in subacute and chronic testing. A much better use of resources than massive blind safety testing is to study the pharmacology of agents that do have effects. If we know what an agent does and how it does it we are in a much better position to determine whether it is affecting exposed humans. Agents showing no selective pharmacological effects at any dose may be thereby eligible for low priority in further testing. An obvious exception to this rule is when large numbers of people are exposed to substantial loads of a substance, especially when the exposure is involuntary. For such an agent intensive safety testing must be performed even if it has to be blind. As effects of more and more agents are described, each new description will occur in a context that will permit it to be seen in perspective and will avoid the furor that currently follows each discovery of toxic effects of a familiar chemical. In a word, any effect will not necessarily be news. Decisions on what actions to take can then be made in a cooler and more rational way. We are seeing perspective develop in the field of carcinogens as a result of the large amount of information being generated by the National Cancer Institute. As more and more substances are found to have some carcinogenic activity, objective appraisal of consequences becomes easier. The National Cancer Institute's results are probably more likely to kill the Delaney Amendment than the Delaney Amendment is to kill saccharine.

The appropriate strategy of OTS would therefore be:

(1) to obtain information on LD50 and on a simple behavioral test (e.g., SMA) in a few mice on as many of the agents to which man will be exposed as possible. It is better to make a few observations of this type on a large number of substances than more extensive observation on a few. Agents with potential hazard in affecting behavior should show a large difference between behavior-affecting doses and lethal doses. Such agents should be referred for more extensive behavioral assessment.

(2) to encourage pharmacological research on potentially toxic agents. Such research should include, in particular, the continued development of more discriminating behavioral tests as illustrated in several papers in this symposium for secondary and tertiary testing and elucidation of mechanisms.

(3) to develop an early-warning reporting system. We should recognize frankly that no laboratory testing system can assure safety even at much less stringent levels than anyone would find acceptable. Therefore, damage must be minimized when a toxic agent is loosed. In retrospect, the number of cases of phocomelia following thalidomide could have been drastically reduced by a relatively simple reporting system that is well within our capabilities.

It is recognized that giving huge doses by unnatural routes may produce effects that need further study before interpretation, but one can work from real effects toward real interpretations while if one is forever groping in the muddy noise of no effect, no real studies are possible. From real effects one can also work toward mechanisms. It is only through understanding mechanisms that we shall be able to have confident predictions of safety. There are too many chemicals to be tested exhaustively individually: we have got to learn enough about molecular toxicology to be able to identify from their chemistry which agents require individual study. Progress in predictive ability is already encouraging [1].

It will be noticed that nothing has been said about the development of short-term in vitro tests for behavioral toxicity, comparable to the mutagenic assays for carcinogenicity. That is because there is little information at present. Insofar as behavior is an emergent function of whole organisms the approach is perhaps less propitious than for other types of function, but the possibility should certainly not be rejected before extensive research on the effects of agents on the behavior of a wide variety of species has been studied, and it is entirely possible that, for example, species of *Arthropoda* or *Mollusca* will provide useful screens for particular types of effects. For neurological toxicity, in vitro assays on cultured neural tissue deserves the interest it is receiving, but, of course, lack of discernable effect of an agent on neural tissue gives no assurance of its innocuousness to behavioral functions.

The Role of Epidemiology

Epidemiology can give us direct fixes on risks at far lower levels than can be attained in the laboratory. Through the study of human populations we may be able to get information on the characteristics of the dose-effect curve at very low levels of effect.

What we need is an agent with a number of special properties. (1) Widely distributed in the human population and at a considerable range of loads, say 100 to 1 or 1000 to 1 differences between individuals. (2) Causing a unique, unequivocal and measurable effect in man and other animal species. (3) A single chemical entity, either not metabolized or with a single metabolic path with inert intermediates and similar in all species. (This requirement rules out cigarettes, which have many desirable features for epidemiological studies.) (4) Easily assayed and having kinetic features such that measurements on urine or on salive faithfully reflect the pharmacologically active load.

If we had such an agent, epidemiological estimates of its effects in the human population should be compared with the dose-effect curve of the agent in two or more species bracketing human sensitivity: that is, in species at least one of which is more sensitive than humans and at least one less sensitive. We can then study the rules for "interpolation from animals to other animals such as man," instead of debating "extrapolation from animals to man" like pre-Darwinian mystics.

Now, of course, at the end of all this labor, all we would have is information on the probable shape of the low end of the dose-effect curve in man and its relation to the middle of the curve in other species for one agent, and perhaps not even an important agent as it will have been chosen for other reasons than its toxicological importance. Is it worth it? Consider the astronomical analogy. It was not until 1837 that the distance to any star was measured. The star in question had very special properties (only one of which was relative nearness) that permitted its distance to be measured. In the ensuing few years, the distances of a few other special stars were measured, then, almost suddenly, when the scale of the universe had been established, other means of determining distances of stars were invented and now the distance of everything is measured almost as a matter of routine. Perhaps we can learn to do the same for astronomically small risks in toxicology.

To return to the laboratory testing of behavioral toxicity the immediate task is to generate more quantitative information on dose-effect curves. Until we have a reasonable body of comparable information we cannot hope to develop general principles of optimum testing, which leads to the last point. The word comparable means being able to be brought together and compared for similarities and differences, the similarities cumulating to a coherent body of information, the differences being further studied. As in most new fields without established practices, the handful of workers in behavioral toxicology have tended to go all their own ways, choosing their agents and methods according to their individual nature and nurture and education and prejudices and funding. The result is that while many different approaches are probed, very little comparable information is accumulated. At this point, as has happened before in new disciplines, people start calling for standardization of methods so more comparable information can be collected, a laudable goal. Again the experience of pharmacology is useful. Attempts to standardize methods have generally not been fruitful, except in rather general terms. Assays in pharmacology are invariably performed with respect to a standard agent rather than by a standard method. There were international standards for drugs such as insulin before active insulin could be reliably purified chemically, the standard being a stable powder with constant insulin activity. Toxicology should follow the same principle. We should standardize with respect to agents. If we agree on agents and loads, then let us each go about assays with all our ingenuity—"let a hundred flowers blossom, let a hundred schools of thought contend" (Mao, 1956)—in the assurance that results will be comparable because of the invariance of agent and load. Thus methods can be compared, better ones selected and improved, poorer ones discarded, all by reference to a constant objective standard.

In conclusion, work establishing dose-effect curves should concentrate on the measurable part of the curve from about 0.1 to 0.9, attempts at direct extrapolation to very low risk should be abandoned as fundamentally unsound and strenuous attempts should be made to link with epidemiology. Many of the points apply to areas of toxicology additional to behavioral toxicology.

We are not ready to recommend batteries of methods and standards in behavioral toxicology to regulating agencies. Indeed, detailed regulations at present would generally be counter-productive. The requirements would be chosen with a high degree of arbitrariness. Most regulatees would happily do the tests, stop thinking or working on the problems, and still more funds would be spent in pursuit of nothing, and ten years from now we would simply know more of what does not happen. With funds devoted to the pursuit of good science, we shall have increasing knowledge with, surely, consequent increase in ability to promote safety. If it is deemed necessary to require some behavioral testing, the requirement should not go beyond the study of a few mice in an objective test such as SMA until we know more. Resources should be dedicated to research, not to routine blind testing.

ACKNOWLEDGEMENTS

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REFERENCES

1. Cramer, G. M. and R. A. Ford. Estimation of toxic hazard—a decision tree approach. *Food and Cosmetic Toxic.* **16:** 225–276, 1978.
2. Dews, P. B. Epistemology of screening for behavioral toxicity. *Environ. Hlth Perspect.* **26:** 37–42, 1978.
3. Dews, P. B. and J. Berkson. On the error of bio-assay with quantal response. In: *Statistics and Mathematics in Biology,* edited by O. Kempthorne, T. A. Bancroft, J. W. Gowen and J. L. Lush. Ames, Iowa: Iowa State College Press, 1954, Chapter 27, pp. 361–370.
4. Dews, P. B. and J. DeWeese. Schedules of reinforcement. In: *Handbook of Psychopharmacology,* edited by L. L. Iversen, S. D. Iverson and S. H. Snyder. New York: Plenum Press, 1977, Vol. 7, pp. 107–150.
5. Ekel, G. J. and W. H. Teichner. An analysis and critique of behavioral toxicology in the USSR. Washington, D.C.: Department of Health, Education and Welfare, 1976.
6. Levine, T. E. Effects of carbon disulfide and FLA-63 on operant behavior in pigeons. *J. Pharmac. exp. ther.* **199:** 669–678, 1976.
7. Palm, P. E., E. P. Arnold, P. C. Rachwall, J. C. Leyczek, K. W. Teague and C. Kensler. Evaluation of teratogenic potential of fresh-brewed coffee and caffeine in the rat. *Toxic. appl. Pharmac.* **44:** 1–16, 1978.
8. Schneiderman, M. A., N. Mantel and C. C. Brown. From mouse to man—or how to get from the laboratory to Park Avenue and 59th Street. *Ann. N.Y. Acad. Sci.* **246:** 237–250, 1975.
9. Wenger, G. R. and P. B. Dews. The effects of phencyclidine, ketamine, *d*-amphetamine and pentobarbital on schedule-controlled behavior in the mouse. *J. Pharmac. exp. Ther.* **196:** 616–624, 1976.

Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function
Neurobehavioral Toxicology, Vol. 1, Suppl. 1, pp. 129–135. ANKHO International Inc., 1979.

Some Problems in Interpreting the Behavioral Effects of Lead and Methylmercury[1]

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LATIES, V. G. AND D. A. CORY-SLECHTA. *Some problems in interpreting the behavioral effects of lead and methylmercury.* NEUROBEHAV. TOXICOL. **1**: Suppl. 1, 129–135, 1979.—Two sets of observations are reported as illustrations of problems encountered in behavioral toxicology. First, in an attempt to determine the contribution of methylmercury-induced ataxia to behavioral changes observed on the fixed-consecutive-number schedule, some ancillary control experiments were undertaken. Neither pharmacologically-produced incoordination (ethanol) nor mechanically-produced incoordination (foot taping) led to behavioral changes similar to those seen after exposure to methylmercury. Second, total crop impaction in a pigeon that died during a behavioral experiment on lead suggested some further work. Lead-induced crop stasis in pigeons was measured by x-raying the passage of force-fed stainless steel ball bearings through the crop. This retardation of motility reliably preceded signs of overt toxicity. These results suggest that the behavioral changes in the pigeon noted by us and reported by other investigators cannot be attributed to CNS dysfunction alone, but more likely arise from starvation, or from combined CNS damage and starvation. In addition, these results demonstrate that the appearance of behavioral effects prior to overt toxicity does not necessarily reflect CNS damage.

Methylmercury Lead Pigeon Fixed consecutive number schedule Ethanol Crop stasis Behavioral toxicology Crop dysfunction Behavior Urecholine

THIS article will describe two sets of observations that illustrate problems that can be encountered by people working in behavioral toxicology. The first concerns one of the side effects of methylmercury in the pigeon, an effect that complicates interpretation of changes in schedule-controlled behavior. The second concerns a serendipitous discovery of one confounding factor that arises during exposure of pigeons to lead.

METHYLMERCURY ATAXIA AND PERFORMANCE ON THE FIXED CONSECUTIVE NUMBER SCHEDULE

A pigeon can be trained to peck on one key a certain number of times and then move over to a second key, peck once, and get food. When the requirement on the first key is specified as no fewer than 8 nor more than 9 pecks, the bird will, indeed, learn to do what is required. Sequences of, for example, 7 or 10 followed by a peck on the second key would not be reinforced, and would lead to a resetting of the response requirement. The bird would then have to start at zero in making its next series of responses. No external stimulus change informs the bird that the requirement has been met, making it a tandem rather than a chain schedule [5]. The sequence of pecks on the first key before the switch to the second is called a *run*. Figure 1 (top left) shows that run lengths of 8 and 9 come to predominate when only those runs lead to reinforcement for the peck on the second key. Figure 1 (bottom left) shows that this particular bird's run length distribution was changed by exposure to methylmercury. Details of exposure are not important for our purposes and are given briefly in the figure legend and in more detail elsewhere (Laties, V. G. and H. L. Evans, in preparation). Suffice it to say here that these are effects of a chronic exposure regimen in which the substance is given in such an amount as to produce a marked effect on behavior in about one or two months, the hope being that we then would be able to follow recovery from this exposure (Fig. 1, top right). The point in the present context concerns the way the run length distribution for this bird shifts to the left, with shorter runs now being emitted before the switch to the second key.

About the time the bird started showing these changes in schedule-controlled behavior, it also started showing some

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[2] Currently supported by a Jr. Staff Fellowship from the National Center for Toxicological Research, Jefferson, Arkansas.

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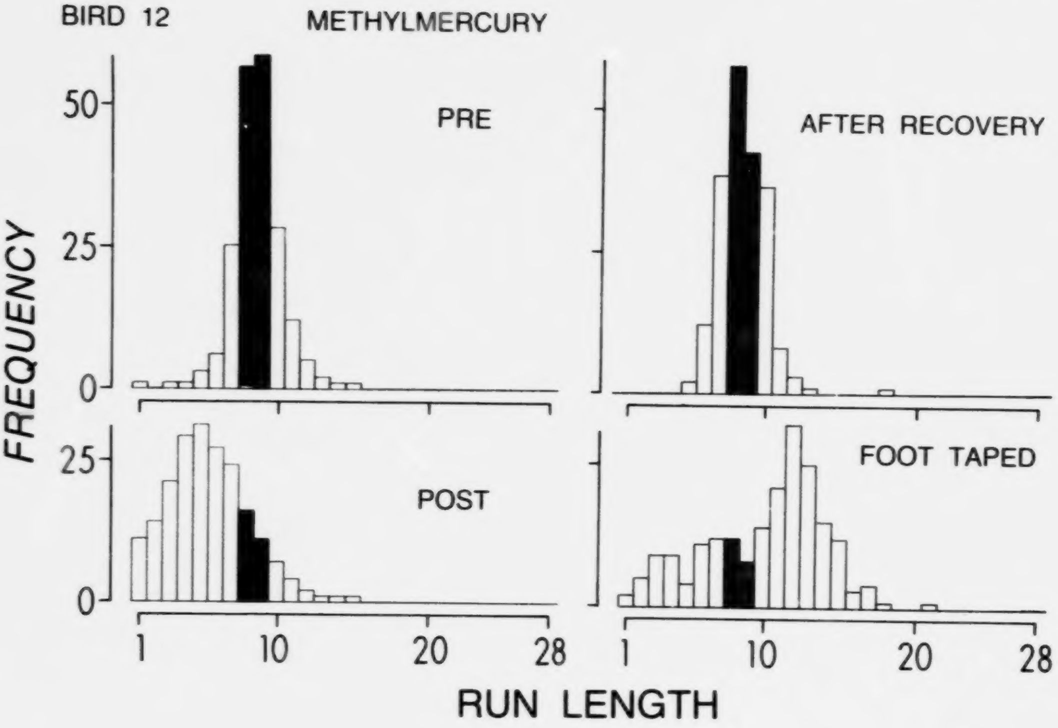

FIG. 1. Performance of a pigeon on the fixed consecutive number schedule of reinforcement. The bird was given 2 mg/kg methylmercury daily Monday through Friday by intubation into the crop several hours after the sessions. Run lengths of 8 and 9 have been filled in. For further details, see Laties, V. G. and H. L. Evans (in preparation).

unsteadiness on its feet and could no longer fly. In order to tease out the effects of the ataxia upon schedule performance, we performed some control experiments to see what ataxia itself could do to this performance.

One manner in which this was accomplished involved binding the large toe of the pigeon back against the shank of the foot, thereby producing a physically, rather than chemically-induced ataxia. This led to the type of performance change shown in Fig. 1, bottom right. Note that the run lengths became longer on the average, an effect opposite to that seen with methylmercury.

More complete data for another bird are shown in Fig. 2, which illustrates how run length was increased greatly during the first foot-taping session but came down somewhat as the pigeon learned how to get around even with one large toe strapped against its leg. Variability was also increased. A measure of the rate during runs is shown at the bottom of the figure. Note that it was not changed by hobbling, even during the first session. In contrast, this rate was invariably reduced by methylmercury (Laties, V. G. and H. L. Evans, in preparation).

We attempted to produce an unsteady gait in another fashion by giving the pigeons ethyl alcohol. The results are shown in Fig. 3 for the three birds studied. Again, the dominant effect of the ethanol, which did indeed produce some unsteadiness in the pigeons, was to increase the number of responses the birds would make on the first key before switching over to the second one and also to increase run length variability. Running rates were unchanged. The results with these two control procedures are summarized in Table 1.

TABLE 1

SUMMARY OF THE RESULTS OF METHYLMERCURY, FOOT TAPING AND ETHANOL ON THE FCN SCHEDULE

	Methylmercury	Foot Taping	Ethanol
% Reinforced	↓	↓	↓
Rate during runs (R/sec)	↓	=	=
Mean run length (resp)	↓	↑	↑
SD of run length (resp)	↑	↑	↑

We would tentatively conclude from these control procedures that the ataxia-producing effects of methylmercury cannot wholly account for the other behavioral effects that we have seen.

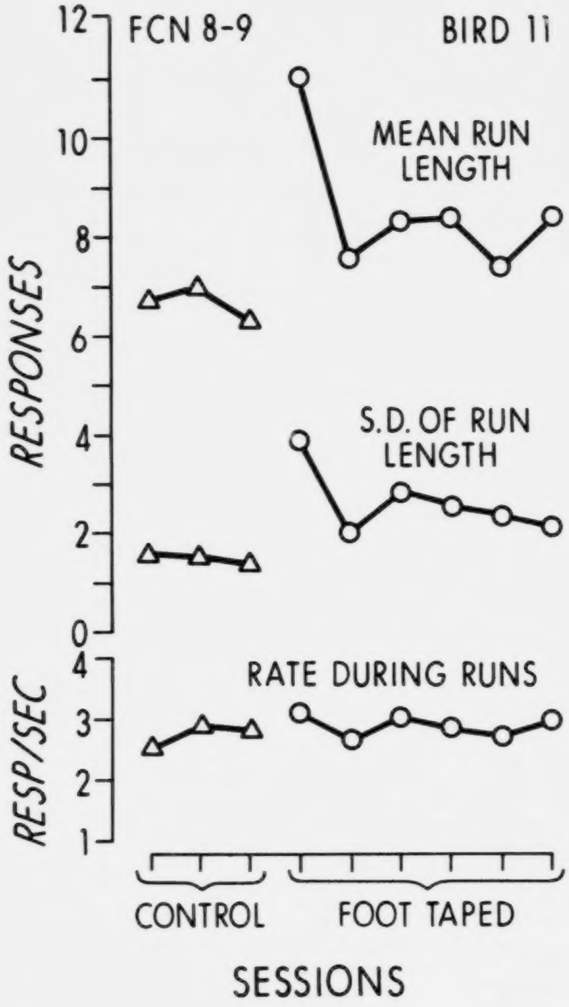

FIG. 2. Effects of foot taping on performance on the fixed consecutive number schedule. For further details, see Laties, V. G. and H. L. Evans (in preparation).

LEAD-INDUCED CROP DYSFUNCTION IN THE PIGEON

In an effort to extend previous investigations [3,11] of lead-induced behavioral changes in rats to the pigeon, several birds were trained on a complex discrimination of temporal duration. Following daily intubations of 12, 36 or 72 mg/kg/day lead acetate, inconsistent behavioral effects, including changes in accuracy and variability of day-to-day performance, a tendency towards color bias, and cessation of responding were observed in the birds. These irregularities in performance were followed by overt signs of toxicity, including very obvious and severe crop dilatation, motor incoordination and regurgitation of crop fluid. Although body weight did not fall, the breast muscle underwent severe atrophy.

Following the death of one severely affected lead-exposed pigeon, gross necropsy revealed that the crop was grossly distended and totally compacted with grit and food particles (Fig. 4). Although the remaining digestive tract appeared normal, no food particles were found below the level of the crop, i.e., the birds appeared to be starving to death. Normally, a sphincter at the base of the crop controls the movement of food boli out of the crop and into the proventriculus or glandular stomach. The system is cholinergically mediated by the vagus. Thus, although we believed we were maintaining our birds' body weights at 80% of ad lib feeding levels, it is obvious that their functional food deprivation levels were much higher. The influence of food deprivation on behavior, has, of course, been extensively documented over the years (e.g., [7,9]).

An identical pattern of toxicity in lead-exposed birds has been reported both by Barthalmus *et al.* [1] and by Dietz *et al.* [4] following proventricular intubation of lead. As with our behavioral effects, changes in pigeons' rates of responding on a multiple Fixed-interval Fixed-ratio schedule following lead exposure by Barthalmus *et al.* [1] were very inconsistent with increases, decreases and no changes reported. Two lead-exposed birds from the Dietz *et al.* [4] investigation actually exhibited decreases in the number of errors on a discrimination task during the latter half of lead exposure. Together with our observations, the ancillary observations of these authors suggest that the behavioral changes noted by them and by us may have arisen not from lead-induced CNS changes, but, from starvation, or, at best, some unknown combination of lead and starvation.

Subsequently, an experiment was undertaken (Cory-Slechta, D. A., R. H. Garman and D. S. Seidman, in press) in an attempt to determine:

(1) the time in days, post-lead-exposure to onset of crop stasis: (2) the blood lead levels at which the effect occurs: (3) the role of intubation as a possible causative factor, and (4) possible histopathological correlates.

To accomplish this, several birds were trained to consume their drinking water from a curved glass calibrated drinking tube to which they had access for approximately two hours a day. On Monday and Thursday of each week, each bird was force fed a 2 mm stainles steel ball bearing; 15 min later an x-ray was taken to ensure the position of the ball bearing in the crop. This was deemed the 0 hr test and the results of one such test are shown in the left half of Fig. 5. Subsequent x-rays were taken 24 hr later (24 hr test) to track passage of the ball bearing out of the crop. Five control determinations were made on each bird, and in every case, the ball bearing had reliably passed out of the crop (and into the gizzard) within 24 hr (Fig. 5, right).

The drinking solutions of the animals were then changed to 0, 1000 or 3000 ppm lead acetate and ball bearing tests continued until increases in crop passage time were observed. At that time, lead exposure was terminated and the birds sacrificed at various times thereafter. An increase in crop passage time is illustrated in the x-rays shown in Fig. 6. In this case, the ball bearing had shown no movement whatsoever within the crop during the 24 hr period.

The effects of lead acetate drinking solutions on days to crop stasis are shown in Fig. 7. Two control birds were exposed to 0 ppm for over 100 days, but showed no change in ball bearing crop passage time and are consequently represented by open circles. The birds exposed to 1000 ppm developed crop dysfunction in 54 and 99 days, whereas both birds exposed to 3000 ppm showed crop stasis within 10

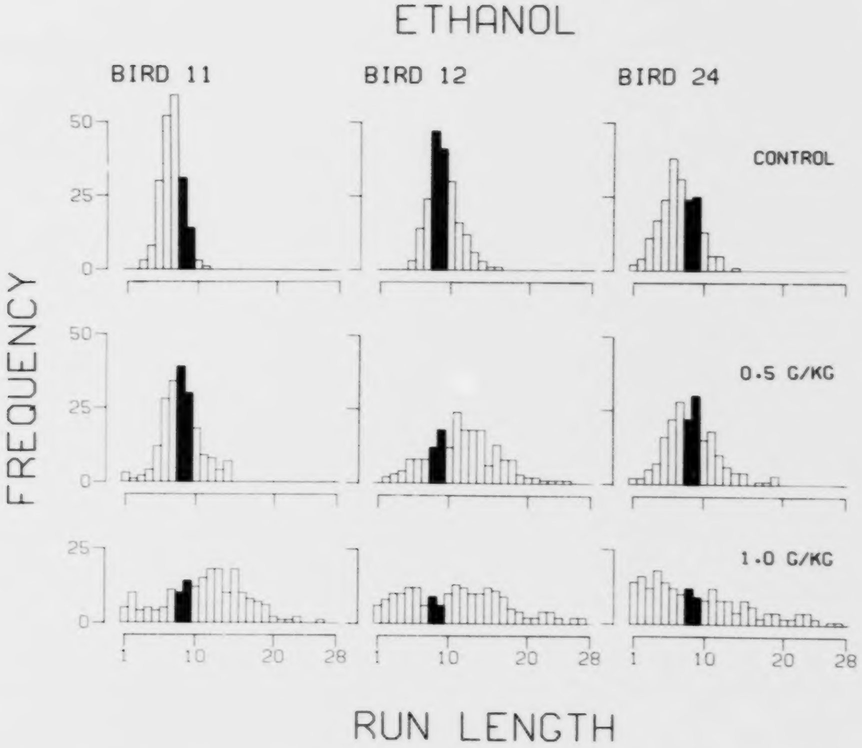

FIG. 3. Effects of ethyl alcohol on performance on the fixed consecutive number schedule. Dosing via intubation occurred 10 min. before the session. The effects of the prior treatment with methylmercury (completed more than a year before) are still evident in the run length distributions of birds 11 and 24. For further details, see Laties, V. G. and H. L. Evans (in preparation).

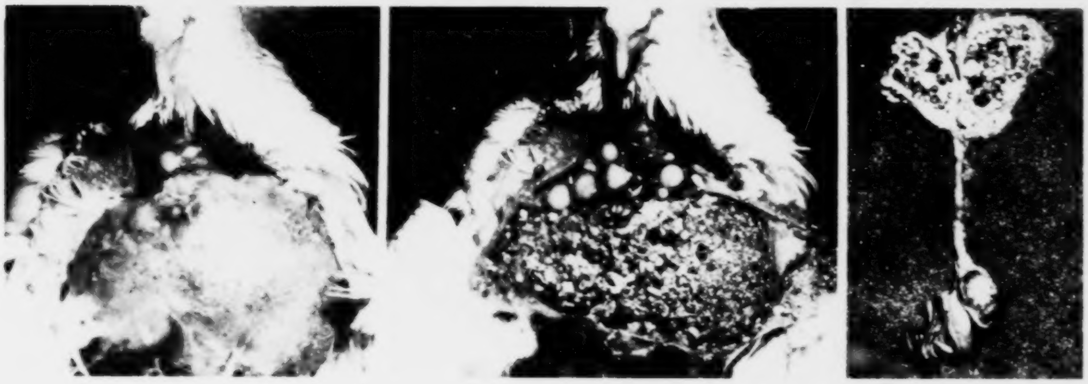

FIG. 4. Photographs depicting the phenomenon of lead-induced crop stasis in the pigeon. Gross necropsy revealed severely distended crops (left) totally compacted with grit and food particles (middle). Although the remaining digestive tract appeared normal (right), no food particles were found below the level of the crop.

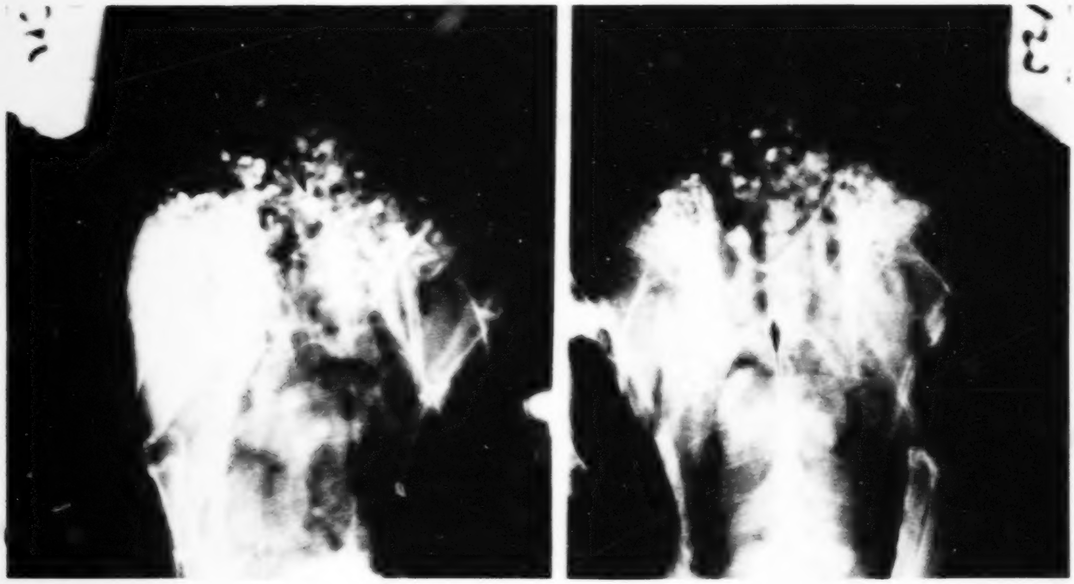

FIG. 5. Demonstration of the determination of crop passage time by the ball bearing test. Birds were force-fed a 2 mm stainless steel ball bearing and radiographs were made 15 min later (0 hr test) to ensure its position in the crop (left). Subsequent radiographs were made 24 hr later (24 hr test) to track passage out of the crop. In this case, as in all control conditions, the ball bearing had reliably passed from the crop to the gizzard within 24 hr (right).

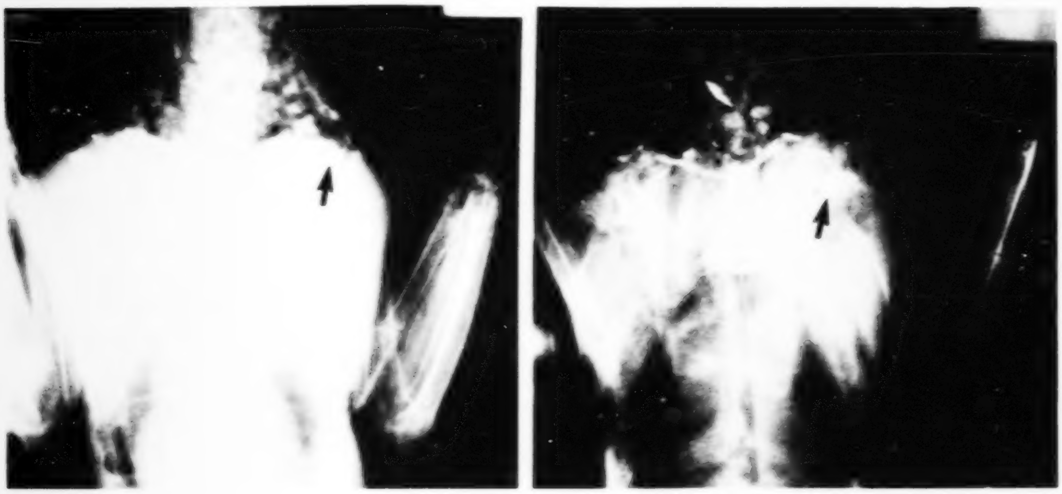

FIG. 6. Demonstration of increased crop passage time as determined by the ball bearing test. Radiographs revealed the stainless steel ball bearing to be located in the crop at 0 hr (left) and to have remained there 24 hr later (right). Subsequent radiographs revealed it to remain immobile as long as 10 days afterwards, when the bird was sacrificed.

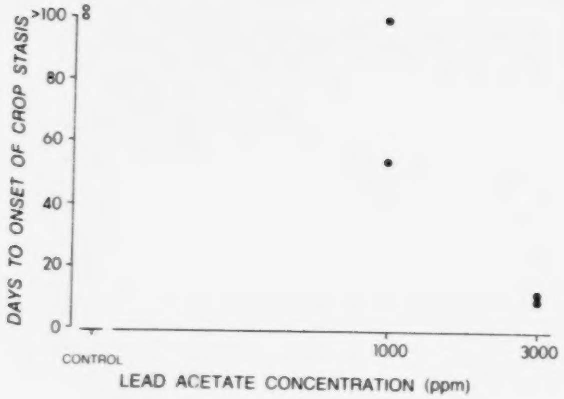

FIG. 7. Time (in days) post-lead exposure to onset of crop stasis as a function of lead acetate exposure concentration. Crop dysfunction was determined by the ball bearing test. Two birds exposed to 0 ppm lead acetate drinking solutions for over 100 days did not show crop stasis and are represented by open circles. The two birds exposed to 1000 ppm showed crop stasis at 54 and 99 days, while both 3000 ppm exposed animals displayed the effect in 10 days.

days. These findings extend the generality of lead-induced crop dysfunction and suggest the phenomenon is not an artifact of crop or proventricular intubation. Additionally, in each case, increases in crop passage time preceded signs of overt toxicity by at least a week (but could have been more since the x-rays were taken only twice weekly), making it both a reliable and sensitive measure. Once the ball bearing stayed in the crop, subsequent x-rays showed it to remain immobilized as long as 10 days afterwards, suggesting that the crop stasis may be an all-or-none phenomenon. To repeat, changes in crop passage time (and thus functional food deprivation levels) were found to precede signs of overt toxicity. It would therefore be a mistake to conclude that behavioral changes seen in the birds prior to overt toxicity are necessarily attributable to CNS effects.

The results of these crop intubations and the proventricular intubation exposures of Barthalmus *et al.* [1] are summarized in Fig. 8. We have not included the results of Dietz *et al.* [4] here due to an apparent inconsistency between the dosing regimen and blood lead levels. These data suggest that proventricular intubation produces overt toxicity at lower concentrations than does crop intubation. Although a dose-effect function is apparent, differences in individual susceptibility are also apparent.

Only one lead-exposed pigeon from this investigation failed to demonstrate overt toxicity. Blood lead analysis showed its levels to fall consistently below 200 μg/dl. Most of

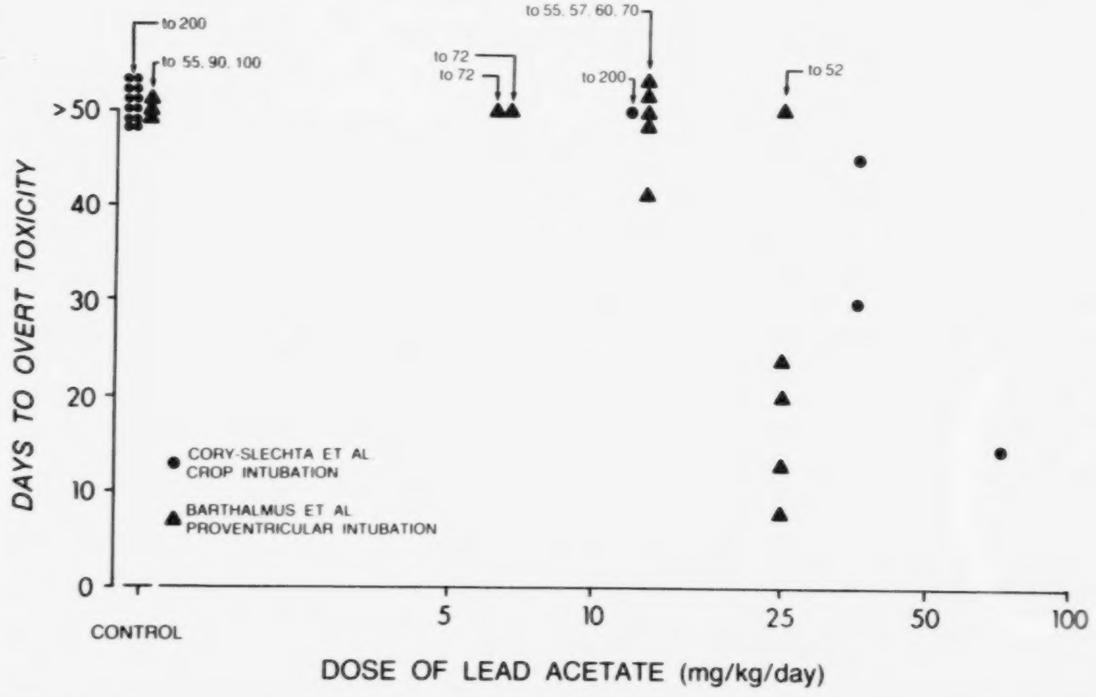

FIG. 8. Days post-lead exposure to onset of overt toxicity as a function of lead acetate exposure dose. Circles indicate birds exposed via crop intubation by Cory-Slechta *et al.* (in press) while triangles represent birds exposed via proventricular intubation by Barthalamus *et al.* [1] Arrows and numbers above symbols represent pigeons who never showed overt toxicity through the number of exposure days indicated. These data suggest that proventricular intubation produces overt toxicity at lower concentrations than does crop intubation. While a dose-effect relationship is suggested, large individual differences in susceptibility are also apparent.

the birds exposed by Barthalmus *et al.*, and all of those exposed by Dietz *et al.* showed blood levels well above 200 μg/dl. However, because of differences in concentration and duration of exposures, route of administration and method of blood lead analysis, further research is needed to delineate more clearly the parameters of lead exposure that result in crop stasis. But, to reiterate, these observations, as well as those of Barthalmus *et al.* and Dietz *et al.*, certainly suggest that the behavioral changes noted by them and by us may have arisen not from lead-induced CNS changes, but from starvation or, at best, some unknown combination of starvation and lead.

No histopathological explanation for the crop stasis emerged from examination of H & E stained tissue (Cory-Slechta, D. A., R. H. Garman and D. S. Seidman, in press). This raises the possibility of a subcellular mechanism, e.g., a decrease in the release of acetylcholine at the neuromuscular junction of the crop sphincter. One severely affected bird was serially treated with urecholine (a parasympathomimetic known to stimulate mainly the GI tract). (Urecholine (bethanechol chloride) from Merck, Sharp and Dohme, West Point, Pa.) This treatment totally cleared the bird's crop, such that within one week, ball bearing passage time through the crop had returned to normal. No lead-exposed bird who was not so treated ever showed any such recovery during the time period we observed them. If one mechanism of lead toxicity does involve disturbances in cholinergic transmission, (e.g., [7,12]) the pigeon might provide a good model for exploring this possibility.

Effects of lead on the digestive system are certainly not unique to avian species. Domestic animals with lead poisoning exhibit various degrees of derangement of the GI tract. Young calves show anorexia and colic, while in sheep the syndrome reportedly consists of anorexia, abdominal pain and, often, diarrhea. At some time during the course of poisoning, approximately 87% of dogs show GI signs consisting of emesis, colic, diarrhea and anorexia [9]. Exposure of lactating rats dams to relatively high concentrations of lead decreased food consumption and necessitated the use of pair-fed control rats [10]. In human adults, the syndrome of acute abdominal colic due to lead poisoning consists of constipation, followed by attacks of crampy diffuse abdominal pain. When pain and constipation are severe, there may be vomiting and anorexia with associated weight loss. In children, the syndrome is similar: colic, anorexia, episodic vomiting and constipation [9].

Whether the effects on the digestive system of these different species involve similar mechanisms is as yet unclear. According to Casarett and Doull [2], these gastrointestinal signs of intoxication are probably related to peripheral neuropathy rather than a direct effect on the intestinal mucosa. The crop impaction of the pigeon reported here thus seems to be among a class of effects of lead on the GI tract, rather than a species specific phenomenon. Such GI changes may well be associated with changes in feeding behavior. The implications so engendered have been largely ignored in studies assessing the effects of lead on food-reinforced behavior.

SUMMARY

Both examples discussed here illustrate the need for great care in interpreting data in behavioral toxicology. Suitable control observations will always be required when the actions of a chemical are complex.

REFERENCES

1. Barthalmus, G. T., J. D. Leander, D. E. McMillan, P. Mushak and M. R. Krigman. Chronic effects of lead on schedule-controlled pigeon behavior. *Toxic appl. Pharmac.* **42:** 271–284, 1977.
2. Casarett, L. J. and J. Doull. *Toxicology: The Basic Science of Poisons.* New York: New York, McMillian, 1975, p. 479.
3. Cory-Slechta, D. A. and T. Thompson. Behavioral toxicity of chronic postweaning lead exposure in the rat. *Toxic. appl. Pharmac.* **47:** 151–159, 1979.
4. Dietz, D. D., D. E. McMillan and P. Mushak. Effects of chronic lead administration on acquisition and performance of serial position sequences by pigeons. *Toxic. Appl. Pharmac.* **47:** 377–384, 1979.
5. Ferster, C. D. and B. F. Skinner. *Schedules of Reinforcement.* New York: Appleton-Century Crofts, 1957, p. 415.
6. Gollub, L. R. and J. T. Urban. The accentuation of a rate difference during extinction. *J. exp. analysis Behav.* **1:** 365–369, 1958.
7. Kostial, K. and B. Vouk. Lead ions and synaptic transmission in the superior cervical ganglion of the cat. *Br. J. Pharmac.* **12:** 219–222, 1957.
8. Laties, V. G. The modification of drug effects on behavior by external discriminative stimuli. *J. Pharmac. exp. Ther.* **183:** 1–13, 1972.
9. National Academy of Sciences. *Lead: Airborne Lead in Perspective.* Report of the committee on Biologic Effects of Atomspheric Pollutants. Washington, D. C., 1972, p. 86, 182–184.
10. Sauerhoff, M. W. and I. A. Michaelson. Hyperactivity and brain catecholamines in lead-exposed developing rats. *Science* **182:** 1022–1024, 1973.
11. Slechta, D. A. The effects of chronic lead acetate administration on fixed interval performance in the rat. Unpublished doctoral dissertation. University of Minnesota, Minneapolis, Minnesota, 1977.
12. Silbergeld, E. K., T. J. Fales and A. M. Goldberg. The effect of inorganic lead on the neuromuscular junction. *Neuropharmacology* **13:** 795–801, 1974.

Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function
Neurobehavioral Toxicology, Vol. 1, Suppl. 1, pp. 137–148. ANKHO International Inc., 1979.

Screening for Neurobehavioral Toxicity: The Need for and Examples of Validation of Testing Procedures

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TILSON, H. A., C. L. MITCHELL AND P. A. CABE. *Screening for neurobehavioral toxicity: The need for and examples of validation of testing procedures.* NEUROBEHAV. TOXICOL. 1: Suppl. 1, 137–148, 1979.—The need for a sensitive and reliable screen to assess environmental agents for potential behavioral and neurological toxicity is discussed. Factors involving strategy, choice of animals and doses, route of administration, duration of study and requirements for the selection of neurobehavioral tests are also evaluated. The primary emphasis concerns the need for standardization and validation of neurobehavioral tests to be used in neurotoxicology. It is suggested that test validation be accomplished by comparing the observed results of known neurotoxicants in animal models which are chosen to predict effects based on reported human symptomatology. As a means of demonstrating how test validation is used in our laboratory, data from a number of experiments concerning the effects of a variety of chemical agents on three measures of motor functioning were discussed. The neurobehavioral effects of acrylamide, and agent known to produce "dying-back" axonopathies, were assessed using separate techniques presumed to measure hindlimb and forelimb functioning and general motor activity. The prediction that acrylamide will first decrease hindlimb functioning, while decreasing forelimb grip strength and motor activity at higher doses, was confirmed. The validity of the hindlimb measurement was supported using a neurotoxicant, carbon disulfide, known to affect motor functioning in a manner similar to acrylamide. The validity of the forelimb technique was shown indirectly using normative data collected from rats of both sexes tested at various ages, i.e., males were stronger than females and grip scores changed as a function of age. The relative sensitivities of the fore- and hindlimb measurements were found to be approximately the same when used to assess the effects of known muscle relaxants, such as phenobarbital and chlordiazepoxide. Finally, it was predicted and confirmed that an environmental agent believed to affect behavior secondarily to effects on other organ systems would affect all measures of motor functioning at approximately the same dose.

Neurobehavioral screening procedures   Factors to be considered in screening
Examples of validation of neurobehavioral tests

THE Environmental Protection Agency (EPA) currently estimates that there are more than 60,000 chemicals in use commercially, while there are approximately 1,000 new chemicals introduced into the environment each year. Such a massive number of chemical entities poses a problem in the implementation of the Toxic Substances Control Act of 1976 (TSCA), which states, among other things, that chemicals should be assessed for their behavioral and neurotoxic effects. In this regard, the lack of a sensitive and reliable screening program for behavioral and neurological toxicity testing is a clear impediment of the effectuation of TSCA [5,21].

FACTORS TO BE CONSIDERED IN DEVELOPING A NEUROBEHAVIORAL SCREEN

With any screening procedure, there are several aspects which must be taken into account. Included in these are strategy, choice of animals, doses, route of administration, duration of study, and requirements for the neurobehavioral tests. These have been discussed in detail elsewhere [5,21] and will be discussed only briefly in this paper.

Screening Strategies

In Chapter 11, Effects on Behavior, in the National Academy of Sciences Report of 1975 [13], a strategy for screening was presented which adopts a sequential scheme that proceeds through a series of increasingly specific, sensitive determinations, guided by the results of preceding, more general tests. For example, after determining lethal doses, an elementary screen for biological effects begins with relatively simple tests capable of reflecting a broad range of impairments, a part of which includes those related to nervous system function. Depending on the effects observed, a substance progresses through a sequence of testing procedures leading to a final decision to accept the substance for marketing or reject it because the risks exceed the predicted benefits. The preliminary screen discussed in the National Academy of Sciences Report consisted of procedures typically used in the pharmaceutical industry to screen for central nervous system activity, such as changes in motor activity, gross observational ratings, alterations in reflex responses, and body weight fluctuations.

In our opinion, the preliminary screening tests with which

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we are familiar and which are used in the pharmaceutical industry are often not appropriate for use in neurotoxicology. A major defect in these procedures is that, other than for pain, tests for sensory and cognitive functioning are almost totally lacking. Yet, a major, early complaint with many environmental toxicants concerns alterations in these neurobehavioral functions. A more complete battery of screening tests should be developed and this issue is discussed in more detail below.

Choice of Animals

The extrapolation of animal toxicological data to man is always tenuous, but for obvious reasons, animals are necessarily used. Unfortunately, there is no single animal model in which effects correlate perfectly with toxicity in humans.

In the preliminary screening of a large number of known or suspected environmental toxins, there are distinct economic factors which must be taken into account. It is also important that there be an adequate pharmacological and toxicological data base for the species chosen for study, such that meaningful interpretations of effects can be made and appropriate hypotheses about mechanisms and loci of action can be framed. For these reasons, the mouse or rat are frequently preferred in the preliminary screen.

Other variables, besides species, must be considered. Efforts should be made in a preliminary screen to minimize the variance of the data. Since the estrous cycle of female animals might introduce additional variance into the data of some behavioral tests [28], male animals are often preferred in the preliminary screen. Another organismal variable is the age of the subject. Variability is generally less in mature adult organisms than in either developing organisms or older, senescent animals.

We are well aware that primate or other species might provide a more sensitive assay than rodents for threshold estimates with many neurotoxicants [15]. Likewise, we are aware of the importance of studying effects of environmental toxins on the young, the old, the malnourished, the sick, females as well as males, etc. in order to determine the population at greatest risk. The purpose of a preliminary screen is not, however, to determine the population at greatest risk. Rather, it is to provide an initial, *tentative* evaluation of the effect of agents on behavior and the nevous system. This can best be accomplished by keeping the experimental variables to a minimim. In effect, we are applying the *Principle of the Blunt Ax* proposed by Lincoln Moses, which states that "if the ax chopped down the tree, it was sharp enough." Moses [10] was pointing out that, if under some circumstances a simple statistical test might demonstrate the reliability of a difference, a complicated analysis would than be a waste of time. If the preliminary neurobehavioral screen results in either the rejection of a substance or in "flagging" it for further (directed) study, it was sharp enough. It is only in those cases where untoward effects are not observerd that the screening ax must be sharpened before approving the substance.

Choice of Doses

The detection of cumulative toxicity following repeated exposure to subthreshold doses is a major goal in the development of a screening program. Thus, a multiple-dosing regimen is particularly appropriate since both quantitative and qualitative changes in the response to environmental factors can occur on repeated exposure, or even with time following a single exposure [5]. A subacute, multiple-dosing regimen used routinely in screening procedures is one which spans about one-tenth of the expected life span of the tested animal [3]. Neurobehavioral assessments should also be made for a time following cessation of the dosing regimen, since it is of interest to determine the permanency of any effects noted during the dosing phase, or to note any delayed effects following cessation of dosing.

Choice of Tests

Two interrelated methodological problems which confront behavioral toxicology as it applies to environmental agents are the insidious onset of effects and the subjective nature of the complaints that are associated with earlier stages of toxicosis. Because of these problems, there is limited agreement as to the sensitivity and utility of many commonly used neurobehavioral tests and procedures. Indeed, Evans and Weiss [5] have stated that "few rigorous animal models are available to substantiate the kind of human symptoms and functional changes that occur with low-level exposure" (to a toxic agent). According to Dews [4] there are no methods that have successfully predicted when prolonged exposure to a low level of an agent will lead to subtle and delayed behavioral effects in man.

The problem of selection of tests for use in neurobehavioral toxicology is not based on the number of tests available, but related to the apparent lack of rational criteria for the choice of suitable methods. Evans and Wiess [5] refer directly to this problem in their statement "too many studies in behavioral toxicology have been purely descriptive, with neither an attack on underlying mechanisms nor a clear extrapolation to human health questions. . . " This, in our opinion, is the major problem with selecting a neurobehavioral test battery. Certainly, chemists do not want to use an instrument which has not been calibrated properly or which is not sensitive to the agent being studied. The situation should be no different for neurobehavioral testing. Thus, the present state of development of behavioral toxicology suggests that methods need to be *standardized* and *validated* before they can be maximally utilized in any neurobehavioral testing program.

Test procedures of unknown utility cannot be validated by testing them against substances producing unknown or controversial effects. The most systematic approach to the validation of sensitive and reliable methodologies for neurobehavioral toxicology is to compare compounds known to have specific neurotoxic effects in a battery of tests chosen to detect a wide range of possible effects and to overlap in terms of signs evaluated. The sensitivity and selectivity of those methods assumed to measure the same neurobehavioral function might be determined by generating a profile of effects that will be characteristic for each compound. Test validation will be achieved by demonstrating the similarities between techniques presumed to measure similar functions and by distinguishing between methodologies assumed to assess different processes.

In the development of the research program at the Laboratory of Behavioral and Neurological Toxicology of the National Institute of Environmental Health Sciences, the first step taken was to identify behavioral methods and representative toxicants to be used in the validation and standardization process [21]. It must be noted that those tests chosen initially will not necessarily be those used ulti-

TABLE 1

SYMPTOMATOLOGY REPORTED BY HUMANS EXPOSED TO NEUROTOXINS

Function Affected	Symptomatology
Sensory	Anosmia
	Paresthesias in feet, fingers, toes
	Visual deficits, photophobia, nystagmus
	Auditory deficits, tinnitus
	Perceptual dysfunctions, pseudohallucinations
Motor	Weakness in hands, arms, legs, paralysis
	Incoordination, dizziness
	Fatigue
	Tremor, convulsions
	Hyperactivity
	Slurred speech
Arousal	Nervousness, irritability, agitation, euphoria, psychosis
	Apathy, lethargy, depression, compulsive behavior
Associative (cognitive)	Impaired short-term memory
	Impaired long-term memory
	Confusion, disorientation
Physiological and consummatory responses	Disrupted sleep-awake cycles
	Hypothermia, hyperthermia, sweating
	Loss or stimulated appetite
	Loss or gain in body weight

TABLE 2

PRIMARY TEST BATTERY

- Sensory Tests
  - Visual Orientation/Localization
  - Auditory Localization
  - Tactile Stimulation Orientation
  - Pain (Tail Flick to Hot Water)
  - Negative Geotaxis
- Motor Tests
  - Spontaneous Motor Activity
  - Forelimb Grip Strength
  - Hindlimb Grip Strength
  - Tremor
- Tests for Arousal Deficits
  - Emergence
  - Startle Response (Air Puff, Auditory Cue)
- Tests for Associative (Cognitive) Functions
  - Rapid Escape/Avoidance Conditioning Test (RE/ACT)
  - Retention
  - Passive Avoidance
- Tests of Physiological and Consummatory Responses
  - Body Weight
  - Rectal Temperature
  - Autonomic Signs
  - Respiration

mately. The final test battery will evolve slowly as knowledge is gained through the validation process.

An adequate neurobehavioral screen should consist of methods which have the potential to predict human toxicosis symptomatology. Thus, those procedures thought to measure deficits analogous to neurological manifestations reported by humans exposed to neurotoxins should be considered. Table 1 contains a list of such symptoms and, as can be seen, they may be grouped into several categories including areas of sensory function, motor strength and coordination, emotionality, associative/cognitive factors and several other effects (e.g., insomnia, anorexia, hyper- and hypothermia).

Table 2 lists the tests that are currently undergoing standardization and validation in our laboratory. These methods, which have been termed the " primary test battery," are grouped according to the category of human toxicosis symptomatology for which they are presumed to measure. All of the procedures are simple to perform and the entire battery takes approximately eight hours for 2 to 3 people to complete and examination of 40 animals. In practice, this is typically done on two successive afternoons. The tests were originally designed for rats, but have in most cases been adapted successfully for mice. Animals receiving chemicals subacutely, as well as chronically, have been tested routinely in this battery and recently the tests have been used to assess mature offspring of mothers exposed to chemicals during gestation. Similar tests for the evaluation of neurobehavioral functioning of animals prior to weaning are now under development.

There is obvious overlap in many of the categories. For

TABLE 3

REPRESENTATIVE NEUROTOXICANTS CLASSIFIED ACCORDING TO MECHANISMS OF ACTION AND PROJECTED FOR USE IN THE VALIDATION PROCESS

General Mechanism of Neurotoxicity	Compound
Agent that produces demyelination	Triethyl Tin
Agent that produces "dying back" neuropathies	Acrylamide
Agents that produce mixed central and peripheral neuropathies	Methyl Mercury Inorganic Lead Arsenic
Agents affecting specific CNS nuclear groups	Salicylates
Agent whose mechanism of neurotoxicity in humans is not yet well defined	Arochlor 1254 Kepone
Pharmacological tools	*d*-Amphetamine

instance, a deficit noted in one of the sensory tests could be associated with a motor impairment, whereas an effect observed in one of the motor tests could be due to decreased responsiveness to all stimulation or general malaise. Nonetheless, an examination of the profile of changes produced by a given substance should provide considerable information about the neurobehavioral system(s) upon which to focus subsequent work.

Neurotoxicants chosen for study were selected according to the symptomatology produced and mechanism of action in humans. As a means of providing a framework for future work it was decided to study representative agents from a standard classification of neurotoxicants, such as that described by Norton [11]. This schema is relevant since it divides toxic agents into general categories based upon symptomatology and mechanism of action. For purposes of comparison between humans and animals, it was decided that adequate information concerning the absorption, distribution, metabolism, and dwell time in the body should be available for both humans and rodents. This severely restricted the number of possible agents (Table 3). In addition to representative neurotoxicants, various psychoactive drugs and/or other experimental manipulations will be used in the test validation process.

EXAMPLES OF THE TEST VALIDATION PROCESS

The remainder of this paper will focus on the validation process and will attempt ot demonstrate how it works in our laboratory. For purposes of illustration, data collected from a variety of studies concerning the motor portion or our neurobehavioral screen will be discussed.

Spontaneously occurring motor activity, forelimb grip strength, and hindlimb extensor responses are used to generate a profile of motor related effects, which is then evaluated to determine the presence or absence of specific motor dysfunction. For example, motor activity can be altered by many factors, central and peripheral, and changes in the frequency of this behavioral measure might be due to a variety of reasons. Thus, it may be regarded as a general or relatively nonspecific, but not necessarily insensitive, indicator of toxicity. Alterations in forelimb grip strength or hindlimb extensor thrust at doses lower than those required to produce changes in general motor activity can indicate the presence of a selective neurotoxic effect. A change in all three measures of motor functioning at the same dose is suggestive of a relatively nonspecific behavioral toxic reaction.

Description of Methods

Hindlimb extensor thrust was assessed using a device reported elsewhere [1]. In this test, a rat is held by the tail and its forelimbs placed on a Plexiglas ledge located approximately 18 in. above the countertop. The hindlimbs of the rat are placed on a T-bar $1\frac{1}{2}$ in. below and 3 in. laterally from the ledge. The T-bar is attached to a strain gauge positioned 45° to horizontal. After sitting in the specified position for approximately 5 sec., an air puff is delivered to the rump of the rat. The deflection on the meter of the strain gauge is taken as a measure of hindlimb thrust. An average of the three highest readings of five trials is taken as the hindlimb extensor response.

Forelimb grip strength was measured using a recording grip meter described in detail elsewhere [2]. Briefly, animals are held by the tail and permitted to grasp a wire ring 45mm in diameter connected to a strain gauge. The force in grams required to pull the subject away from the wire ring is measured in three trials, not counting those in which it holds the ring with only one forepaw or jerks the ring. An average of three readings was taken as the grip strength score.

Spontaneous motor activity was measured in commercially available activity monitors (Automex, Columbus Instruments, Columbus, OH) placed inside a sound-and light-attenuated outer chamber. At the beginning of the test, rats are placed individually into one of six plastic cages (11 × 7 × 5 in.) having perforated stainless steel lids and placed on the activity measuring device. General motor activity was measured in darkened conditions over a 9 min period.

All behavioral testing was done on a blind basis so as to eliminate bias in the interpretation of responses. In addition, testing was done between the hours of 10 a.m. and 2 p.m. to control for diurnal variations.

In all of the following studies, the data were analyzed for overall treatment effects using analysis of variance (ANOVA) techniques [29]. If a significant treatment effect was observed, independent group comparisons were made using Fisher's Least Significant Difference (LSD) test [8]. The accepted level of significance in all cases was $p<0.05$.

Effects of a Known Neurotoxin on Motor Functioning

One agent chosen for our validation studies was acrylamide. Repeated exposure to this chemical results in a progressive bilateral polyneuropathy and this agent has been used extensively to study "dying-back" polyneuropathies [18,19]. Acrylamide initially affects distal portions of larger diameter nerve fibers, while with more prolonged exposure, the fiber degeneration progresses, affecting more proximal regions. Sensorimotor functioning of the lower extremities is characteristically affected before that of the upper extremities, while more prolonged exposure affects function to a greater extent. Regeneration of peripheral nerve fibers and restoration of function usually occurs following cessation of exposure [18].

The purpose of the following experiment was to determine the relative sensitivity and selectivity of the hindlimb extensor measure to the effects of acrylamide on motor

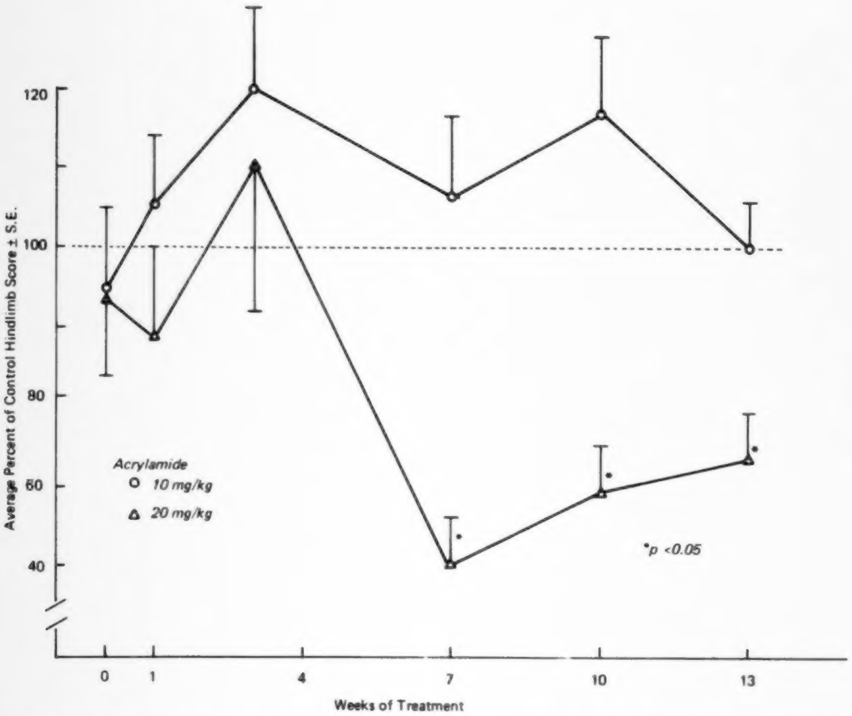

FIG. 1. Effects of acrylamide given orally, 3 times weekly, in doses of 10 or 20 mg/kg on the hindlimb extensor response (HLER) of rats at 0 (predosing), 1, 4, 7, 10, and 13 weeks of dosing. HLER scores of acrylamide-treated rats (N = 10 per group) were expressed relative to the combined average of the two control groups (N = 20) and data are average percentages of control ± S.E. Analysis of variance for repeated measures was used to assess the effects of treatment and time on absolute HLER scores. The asterisks indicate a significant difference between the averages of the combined HLER scores of the control and treated rats (Fisher's Least Significant Difference Test, $p < 0.05$). From Tilson *et al.* [26].

function. On the basis of the literature reviewed above, it was predicted that acrylamide will affect hindlimb functioning before the forelimb grip or motor activity. Upon more prolonged exposure, it was predicted that all three measures of motor functioning will be affected. Furthermore upon cessation of dosing, recovery of function should be observed.

The animals used in this study were male, albino rats of the Fisher-344 strain and weighed approximately 250–300 g at the beginning of the study. Details concerning housing and maintenance conditions and other details of this experiment have been described in detail elsewhere [26]. The present study lasted a period of 120 days, 90 days of which consisted of the dosing phase followed by a 30 day post-dosing period. Rats (ten per group) were randomly assigned to three treatment groups and two control groups. Those animals assigned to the treatment groups received 10 or 20 mg of acrylamide/kg of body weight. One control group received 1ml/100 g of distilled water vehicle (Vehicle Control Group)while the other was not dosed (No Dosing Control Group). No difference between the two control groups were observed and their data were combined to form a single control group for comparison with acrylamide treated animals.

Acrylamide dissolved in water vehicle was given by gavage three times a week for 13 weeks. The behavioral measurements were taken in a blind fashion during weeks 0 (Predosing), 1, 4, 7, 10, and 13 of dosing. Five animals that had received 20 mg/kg and 5 control rats were randomly selected for retesting at 1 and 5 weeks postdosing.

Acrylamide produced a significant effect on the hindlimb extensor response of rats (Fig. 1). Comparisons between means of the treatment and control groups showed a statistically significant decrease in the response at the 20 mg/kg dose beginning on week 7 and continuing throughout the remainder of the dosing period. Following cessation of dosing, the hindlimb scores of the acrylamide animals were significantly lower than those of the controls, but there were no differences between groups 5 weeks after cessation of dosing (Fig.2).

The spontaneous motor activity of rats exposed to acrylamide was also decreased significantly by repeated dosing with acrylamide (Fig. 3). Pairwise comparisons of individual treatment and control means showed a statistically significant decrease in activity at the tenth and thirteenth week of dosing at 20 mg/kg. As in the case of the hindlimb extensor response, motor activity of the acrylamide animals (20 mg/kg group)was still decreased significantly one week after cessation of dosing, while no differences were observed 5 weeks after cessation of dosing (Fig. 4).

Although motor activity and hindlimb extensor responses were affected by acrylamide, no significant effects on forelimb grip strength were observed (Fig. 5). However, one week after cessation of dosing, the forelimb scores of the

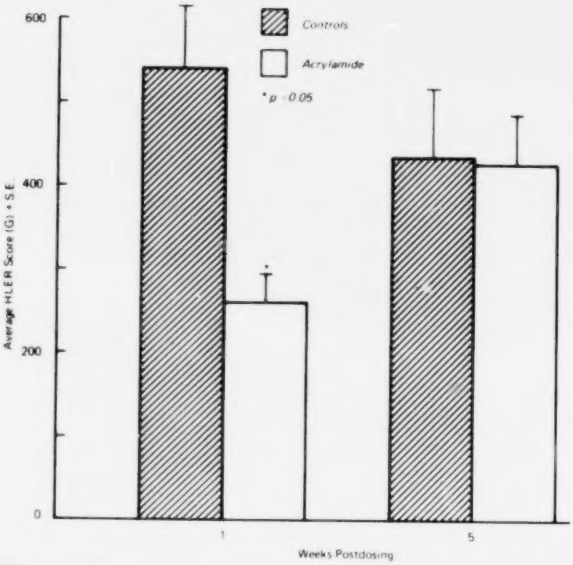

FIG. 2. Average hindlimb extensor (HLER) scores ± S.E. of rats given 20 mg/kg of acrylamide (N=5)or control treatment (N=5) for 13 weeks and randomly selected for retesting at 1 and 5 weeks after cessation of dosing. The asterisk indicates a significant difference between groups (*t*-test,$p<0.05$). From Tilson *et al.* [26].

acrylamide treated animals (20 mg/kg group) were significantly depressed (Fig. 6). There were no significant differences between groups 5 weeks after cessation of dosing.

These results are in accord with the predictions made prior to the beginning of the experiment. Hindlimb function was affected at a cumulative dose of acrylamide lower than that required to affect forelimb grip strength or spontaneous motor activity. More prolonged exposure to acrylamide affected motor functioning to a greater extent. Furthermore, recovery of function was observed following cessation of dosing. Finally, these data are similar to those of a previous report from our laboratory showing hindlimb functioning of rats was affected before forelimb grip strength and motor activity by acrylamide given 5 days per week for one month [22]. Recovery of function was also observed following cessation of dosing.

Effects of Carbon Disulfide on Motor Functioning of Rats

In the preceding experiment, the prediction that acrylamide will affect functioning in the hindlimbs prior to affecting that in the forelimbs was confirmed. The purpose of the next study was to determine the relative selectivity and specificity of the behavioral methods using another agent that produces a profile of neurotoxicity similar to that of acrylamide. The agent chosen for investigation was carbon disulfide (CS_2), since this chemical has been shown in various neuropathological and neurophysiological studies to affect

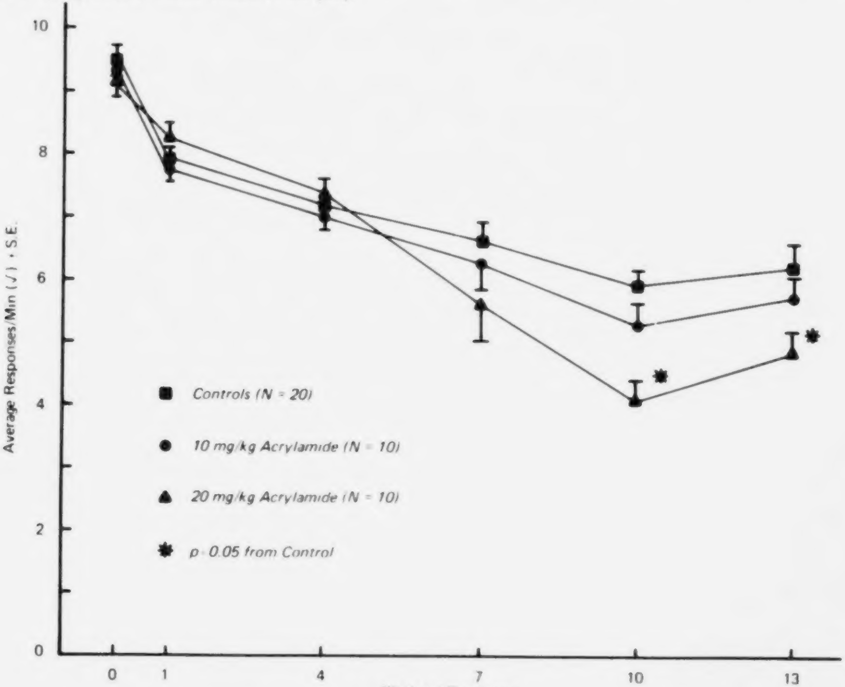

FIG. 3. Effects of 10 or 20 mg/kg of acrylamide given orally, 3 times per week, on spontaneous motor activity of rats tested at various times during the dosing regimen. Activity counts occurring during a 9 min period were converted to responses per min and square root transformed. Data are averages ± S.E. of 10 animals per treatment group and 20 rats in control groups receiving either 1ml/100g of distilled water vehicle or no treatment. Analysis of variance for repeated measures was used to assess overall effects of treatment and time. The asterisk indicates a significant difference between the mean of the combined control groups and the treatment group (Fisher's Least Significant Difference Test, $p<0.05$). From Tilson *et al.* [26].

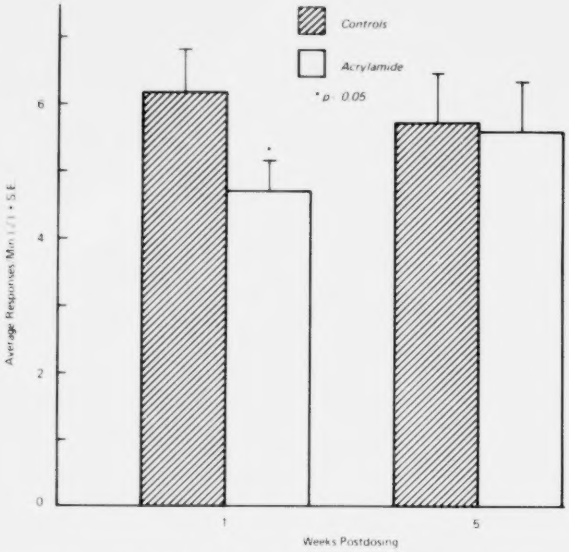

FIG. 4. Average activity counts (expressed as responses per min and square root transformed) ± S.E. of rats given 20 mg/kg of acrylamide (N = 5) or control treatment (N = 5) for 13 weeks and randomly selected for retesting at 1 and 5 weeks after cessation of dosing. The asterisk indicates a significant difference between groups (*t*-test, $p < 0.05$). From Tilson *et al.* [26].

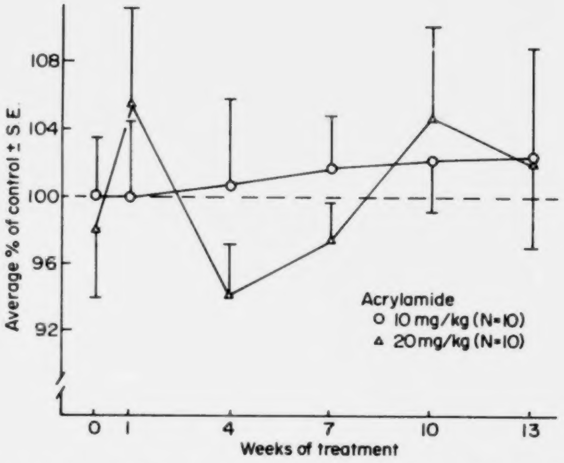

FIG. 5. Average grip scores ± S.E. of rats given 10 or 20 mg/kg of acrylamide orally, 3 times weekly for 0 (predosing), 1, 4, 7, 10, and 13 weeks (N = 10 per group). Controls were dosed with distilled water vehicle (N = 10) or given no treatment (N = 10). Analysis of variance for repeated measures was used to assess overall effects of time and treatment. Asterisks indicate a significant difference between the means of the combined controls (N = 20) and the treatment group (Fisher's Least Significant Difference Test, $p < 0.05$). From Tilson *et al.* [26].

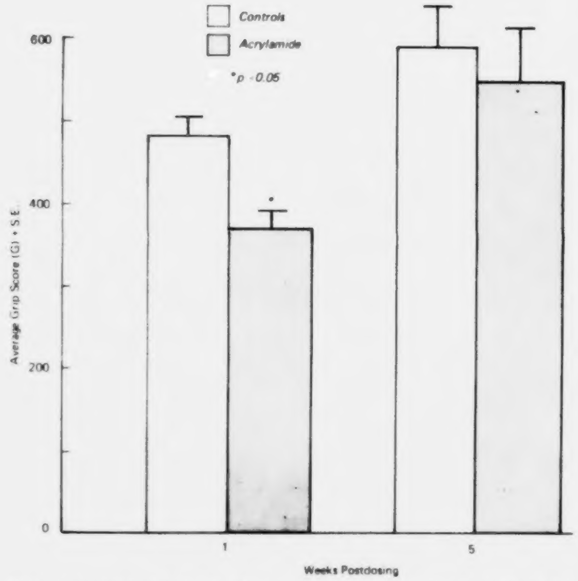

FIG. 6. Average forelimb grip scores ± S.E. of rats given 20 mg/kg of acrylamide (N = 5) or control treatment (N = 5) for 13 weeks and randomly selected for retesting at 1 and 5 weeks after cessation of dosing. The asterisk indicates a significant difference between groups (*t*-test, $p < 0.05$). From Tilson *et al.* [26].

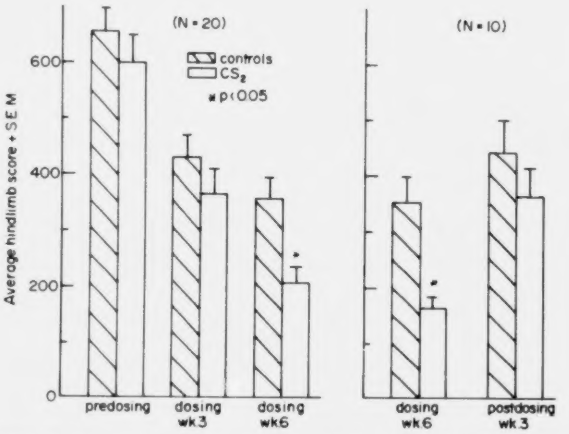

FIG. 7. The effects of CS_2 exposure on the hindlimb extensor response of rats. Data are average (+ S.E.M.) hindlimb scores of animals (N = 20 per group) prior to (PreDosing) and 3 or 6 weeks after inhalation exposure to CS_2 or vehicle (left graph). Data in the right graph are average hindlimb scores of 10 animals in each group after 6 weeks of dosing and 3 weeks after cessation of dosing. The asterisk indicates that the treated group differs significantly from vehicle exposed animals (Fisher's Least Significant Difference Test, $p < 0.05$). From Tilson *et al.* [24].

TABLE 4

EFFECTS OF CS$_2$ ON FORELIMB GRIP STRENGTH OF RATS

Time of Testing	N	Average Grip Score ± SEM Control	CS2
Predosing	20	466 ± 11	482 ± 13
Dosing Week 3	20	481 ± 15	519 ± 10
Dosing Week 6	20	520 ± 15	502 ± 10
Dosing Week 6	10	520 ± 22	499 ± 9
Postdosing Week 3	10	552 ± 12	542 ± 21

From Tilson *et al.* [24]

TABLE 5

EFFECTS OF CS$_2$ EXPOSURE ON LOCOMOTOR ACTIVITY OF RATS IN A NOVEL ENVIRONMENT

Time of Testing	N	Average Counts per 9 Min ± SEM Control	CS2
Predosing	20	709 ± 31	706 ± 24
Dosing Week 3	20	386 ± 30	433 ± 28
Dosing Week 6	20	283 ± 18	250 ± 23
Dosing Week 6	10	302 ± 31	253 ± 50
Postdosing Week 3	10	451 ± 35	426 ± 42

From Tilson *et al.* [24]

the functioning of lower extremities to a greater extent than that in the upper extremities [16, 20, 27].

Male, albino rats of the Fisher strain were exposed to 2 mg of CS$_2$/l of air or air for 4 hours per day, 5 days per week for 6 weeks (24). Twenty rats received CS$_2$ while 20 served as air controls. The neurobehavioral functioning of rats was assessed in the week prior to dosing (Predosing) and after 3 and 6 weeks of dosing. Behavioral tests were administered at least one our after removal of the rats from the inhalation chambers. After cessation of exposures, ten rats from each group were randomly chosen to be retested for recovery of function 3 weeks later.

The hindlimb extensor response decreased significantly after treatment with CS$_2$ (Fig. 7). After 6 weeks of dosing, CS$_2$ treated rats showed significantly lower hindlimb scores than controls. In addition, 3 weeks after cessation of dosing, CS$_2$ treated rats did not differ from controls, indicating a recovery of function. On the other hand, forelimb grip strength was not significantly affected at any time during dosing with CS$_2$, nor were there any effects noted following cessation of dosing (Table 4). Motor activity occurring in a novel environment was also not affected at any time by CS$_2$ (Table 5).

As predicted, CS$_2$ produced a significant decrease in the hindlimb functioning of rats. While effects on forelimb grip strength and motor activity were not observed during the course of the repeated dosing regimen, these effects might be expected to occur had dosing with CS$_2$ continued for a longer period of time. The profile of neurotoxicity observed with CS$_2$ in this study is similar to that observed with acrylamide in the previous experiment and further demonstrates the relative sensitivity of the hindlimb procedure.

Validation of the Forelimb Procedure

The experiments discussed thus far were designed to demonstrate the validity of the hindlimb extensor technique. However, it must be remembered that the three tests of motor functioning are all part of a profile and before the hindlimb technique can be shown to be a specific measure of hindlimb dysfunction, the relative sensitivity and specificity of the forelimb grip procedure must be demonstrated.

The most direct way to show the specificity of the forelimb grip measurement would be to assess a chemical that affects the functioning of the upper extremities prior to affecting functioning in the lower extremities. Seppalainen [15], for example, has reported that motor conduction velocities of nerves in the upper extremities (ulnar and median nerves) were slowed in lead exposed workers, but the nerve velocities in the lower extremities were not significantly influenced. These neurophysiological data correspond well to the observation made by Goldstein *et al.* [6] that in adults, functioning of the upper limbs is affected by lead to a greater extent than in the lower limbs. The generality of this differential effect of lead on the functioning of upper and lower limbs of rats has not yet been demonstrated, although this work is scheduled to be done in our laboratory in the near future.

In spite of the lack of direct evidence to demonstrate the relative selectivity of the hindlimb and forelimb procedures, the validity of the forelimb procedure can be inferred by data from other types of experiments. For example, the forelimb grip strength of rats should be correlated to some degree with the size of the animal, i.e., older animals should have higher grip scores than younger animals. Moreover, if the grip score is dependent upon body weight, then similarly aged animals, having different body sizes, should differ accordingly in their grip scores, i.e., males should be stronger than similarly aged females.

Data from a number of studies conducted in our laboratory on control male and female rats tested at different ages were assimilated to generate Fig. 8. As can be seen, the grip scores of male and female Fisher strain rats increase from day 20 to day 230 of age. In addition, male rats score higher than females beginning at about 120 days of age. It is our experience that male Fisher rats weigh significantly more at that time than the female rats (Squibb, unpublished observations).

Another way of demonstrating the validity of the forelimb technique is to determine the relative sensitivities of the hind- and forelimb procedures using psychoactive agents having known muscle relaxant properties. If the two procedures are, in fact, measuring similar components of muscle exertion in the upper and lower extremities, then the forelimb procedure should be at least as sensitive as the hindlimb technique to the effects of these drugs.

Male rats of the F-344 strain weighting approximately 250 g at the beginning of the study were randomly assigned to receive either 0, 3, 9, or 27 mg/kg of chlordiazepoxide or 0, 20, 40 or 80 mg/kg Na Phenobarbital intraperitoneally 30 min prior to testing hindlimb and forelimb function. There were ten rats per each treatment group.

Analysis of variance indicated that chlordiazepoxide and

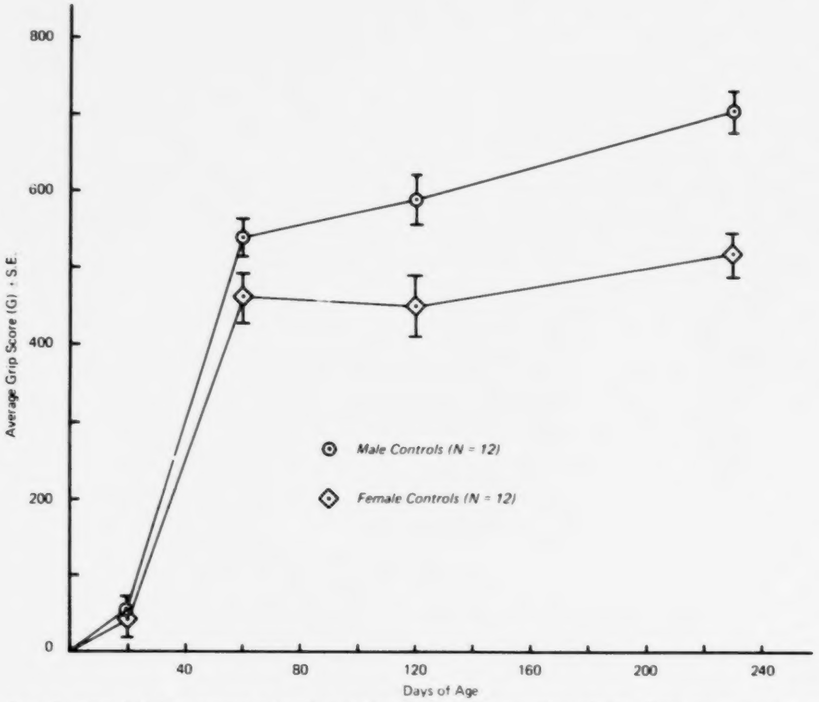

FIG. 8. Forelimb grip scores of male and female rats as a function of age. Data are average grip scores (g) ± S.E. of 12 rats per point. Control male and female rats from 4 different experiments conducted at 20, 60, 120 and 230 days of age were used to construct the curve. The grip scores of the males are significantly more than the females at 120 and 230 days of age (*t*-test, $p<0.05$).

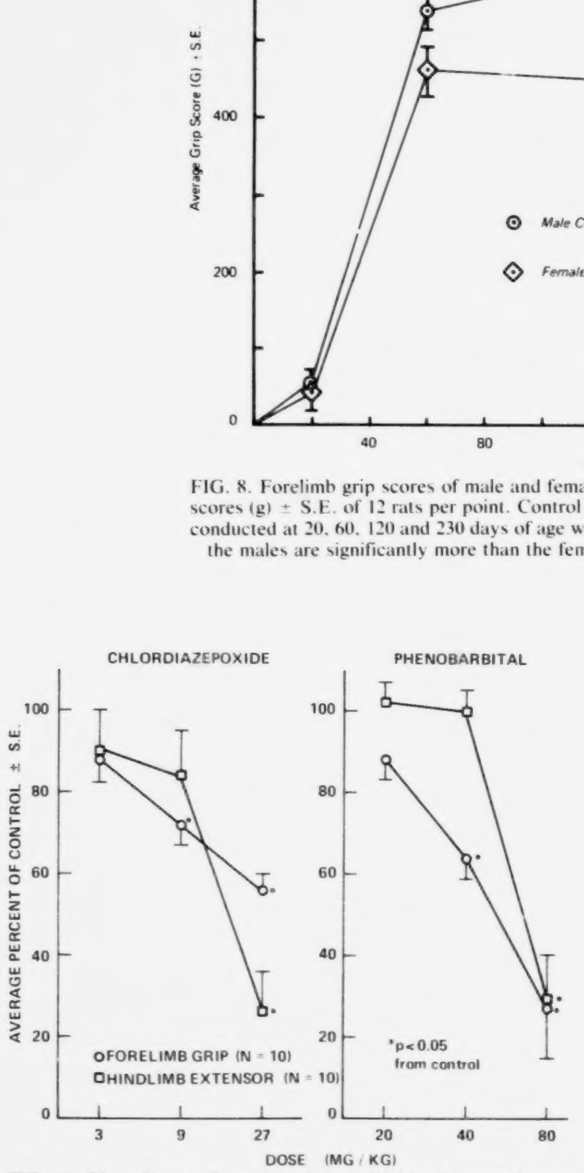

FIG. 9. The effects of various doses of chlordiazepoxide and phenobarbital on the fore- and hindlimb scores of rats. Each point is the average percentage ± S.E. of control (0 mg/kg). There were 10 rats tested at each dose. The asterisks indicate that the mean of the drugged group differed from that of the vehicle control group (Fisher's Least Significant Difference Test, $p<0.05$).

phenobarbital significantly affected the motor functioning of rats as measured by the fore- and hindlimb test (Fig. 9). Those rats receiving 9 and 27 mg/kg of chlordiazepoxide had forelimb grip scores that were significantly lower than controls, while the effect at 3 mg/kg was not statistically significant. The same animals receiving chlordiazepoxide and tested for hindlimb dysfunction also showed decreased responses. However, only the effect at 27 mg/kg was statistically significant. Those animals receiving phenobarbital had significantly decreased forelimb grip scores at 40 and 80 mg/kg, while the hindlimb measure was affected by the 80 mg/kg dose only. Phenobarbital had no effect on fore- and hindlimb scores at any other doses.

The results of these experiments show that the forelimb measure was as sensitive as, if not more sensitive, than the hindlimb technique to chlordiazepoxide and phenobarbital. That the two procedures were affected in a similar fashion by psychoactive agents having muscle relaxant properties not only demonstrates the relative sensitivity of the two techniques, but supports the specificity of the hindlimb deficits observed in the acrylamide and CS_2 experiments.

Example of Putative Nonspecific Neurobehavioral Toxic Reaction

The concept of a profile of effects generated by testing known neurotoxins in a battery of tests having different, but overlapping components, was generally supported in the ac-

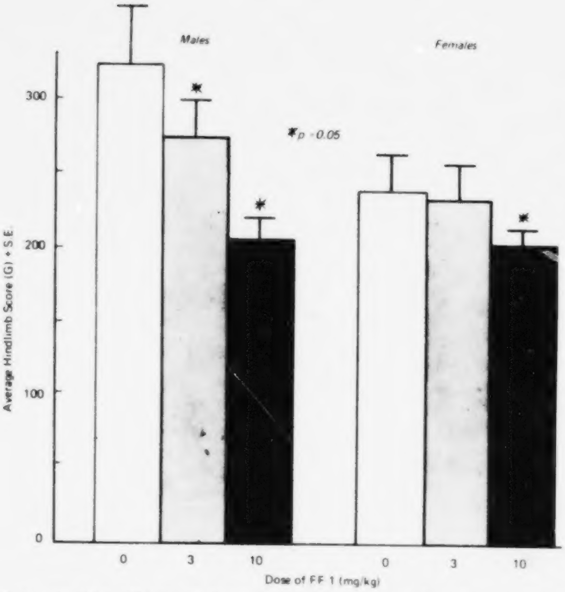

FIG. 10. The effects of FF-1 given to male and female rats for 6 months on hindlimb extensor response. Data are average hindlimb scores (g) ± S.E. Asterisk indicates a significant difference from vehicle treated control rats of the same sex (Fisher's Least Significant Difference Test, $p<0.05$). From Tilson and Cabe [23].

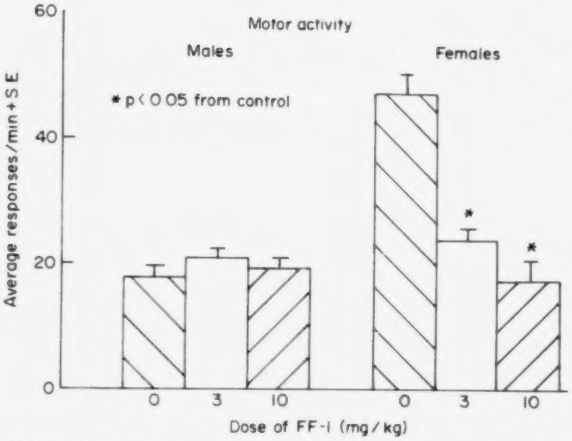

FIG. 12. The effects of FF-1 given to male and female rats on spontanous motor activity. Data are average responses occurring per min ± S.E. in a 9 min test. The asterisks indicate that the mean of the treated group differs statistically from that of the vehicle treated animals (Fisher's Least Significant Difference Test, $p<0.05$). From Tilson and Cabe [23].

rylamide and CS_2 studies. In those experiments, it was predicited that hindlimb functioning would be affected prior to spontaneous activity and forelimb grip strength. The purpose of the present study was to examine the neurobehavioral profile of an environmental agent that might affect behavior secondary to effects on other organ systems.

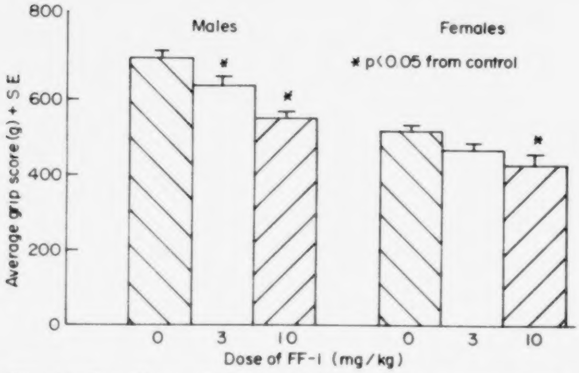

FIG. 11. The effects of FF-1 on the forelimb grip strength of male and female rats treated with FF-1 for 6 months. Data are average forelimb scores (g) ± S.E. The asterisk indicates a significant difference from vehicle control rats of the same sex (Fisher's Least Significant Diffence Test, $p<0.05$). From Tilson and Cabe [23].

Polybrominated biphenyls (PBBs) have been shown to induce hepatic microsomal enzymes and produce liver toxicity in laboratory animals [14,17] and alterations in the functioning of these and other organ systems occurs in doses lower than those required to produce measurable behavioral changes [7, 9, 25]. These effects in laboratory animals and the value symptomatology reported by humans exposed to PBBs [30] suggest that they might influence behavior secondarily to effects on non-neural systems. If the PBBs influence behavior secondarily or affect motor functioning nonspecifically, then it is predicted that they will depress all three measures of motor functioning and these effects will be observed at about the same dose.

Male and female rats of the F-344 strain were used as described elsewhere [23]. A commercial mixture of PBBs, Firemaster FF-1, was suspended in corn oil vehicle and given by gavage, five days per week for a total of 130 doses. There were 7–8 animals of each sex given 3 or 10 mg/kg per dose, while 9 females and 15 males served as controls (corn oil vehicle). The animals were tested for neurobehavioral dysfunction after 1, 2, 4, and 6 months of dosing. Since no effects on behavior were observed after 4 months of dosing, only data collected at the 6 month test are reported.

Chronic exposure to PBBs had a significant effect on the hindlimb extensor response of rats (Fig. 10). The males were more consistently affected by PBBs than the females. Independent group comparisons showed that males were affected significantly by 3 and 10 mg/kg. PBB exposed female rats had lower hindlimb scores than controls at 10 mg/kg but the effect at 3 mg/kg was not significant.

As in the case of the hindlimb measure, the forelimb grip scores of PBB treated rats were decreased after 6 months of dosing with PBBs (Fig. 11). Independent group comparisons indicated that male rats were significantly affected by 3 and 10 mg/kg of PBBs. Female rats receiving 3 mg/kg had lower scores than controls but were not affected significantly, while those rats receiving 10 mg/kg had significantly reduced grip scores.

The effects of PBBs on motor activity depended on the baseline level of activity (Fig. 12). Female rats were much more active than males and 3 and 10 mg/kg of PBBs signifi-

TABLE 6

PREDICTED NEUROBEHAVIORAL EFFECTS OF ACRYLAMIDE IN RATS AT VARIOUS TIMES DURING DOSING*

	Shorter Term/ Lower Dose	Longer Term/ Higher Dose	Recovery
Sensory			
Visual	0	–	–
Auditory	0	0	0
Olfactory	0	0	0
Tactile	–	–	0
Pain	0	0	0
Vestibular	0	0	0
Motor			
Spontaneous Motor Activity	0	–	0
Forelimb Function	0	–	0
Hindlimb Function	–	–	0
Tremor	0	+	0
Muscular Endurance	–	–	–
Arousal			
Emergence	0	0	0
Startle Response	0	0	0
Learning			
One-Way Active Avoidance	0	0	0
Retention	0	–	–
Physiological Functioning			
Body Weight	–	–	0
Core Temperature	0	0	0
Autonomic Signs	+	+	0

*Response: + = measure is predicted to be elevated or rate increased; – = predicted to be decreased in magnitude or rate decreased; 0 = no significant alteration in the measure is expected at the time of testing.

cantly decreased the motor activity of the females. The male rats were not affected significantly by either dose of PBBs.

In summary, the neurobehavioral effects of the PBBs depend on the rate or magnitude of the behavioral dependent variable. If the rate or magnitude of responding is high, then the PBBs decrease that behavior. That the behavioral effect observed with the PBBs was consistent in each of the 3 tests and occurred at approximately the same dose is the type of neurobehavioral profile indicative of a nonspecific toxic reaction. Studies to confirm the generality of such a profile using another agent that produces liver toxicity, such as carbon tetrachloride [12], are planned.

SUMMARY AND CONCLUSIONS

In an attempt ot demonstrate how test methods in behavioral toxicology can be standardized and validated, specific examples concerning three motor tasks used in our laboratory have been discussed. Obviously, a great deal of work will be required to standardize and validate all the tests used in a neurobehavioral test battery such as that described in Table 2. However, the requirement for behavioral and neurological assessment of a large number of chemical substances only accentuates the need to conduct such research.

It is our contention that the success of the validation scheme proposed in this report depends on the predictions made prior to the beginning of the study. Such predictions permit test validation by confirming the usefulness of any given procedure for the type of neurobehavioral symptom and representative chemical being assessed. With increasing probability of success in making such predictions, the utility of behavioral and neurological methods also increases.

It is important to indicate that projections which are not confirmed are as valuable, if not more so, than correct predictions. Failure to confirm expectations for a given substance and procedure exposes the need for additional information about the animal model in question. The data obtained in validation studies of this type can only assist in the development of other more refined techniques. Moreover, such information can provide the basis for reevaluation or reassessment of the mechanism of action of the agent under investigation.

Another important feature of a test validation scheme as proposed in this report is the profile of effects generated for each substance. The pattern of correct and incorrect projections provides a basis for evaluating the competence of the testing systems. Assessment of the profiles resulting from the study of representative neurotoxins can lead eventually to reevaluation of the principle factors underlying the animal model.

For the purposes of illustration, Table 6 contains predicted behavioral effects for acrylamide using the test battery described in Table 2. Based upon a review of the human neurotoxicology of acrylamide [12,19], a symptomatological profile was generated. The pattern of expected effects for acrylamide was then generated for the neurobehavioral test battery currently used in our laboratory. Table 6 summarizes these predictions in terms of the presence or absence of an effect, the direction of the effect, and whether or not the effect can be reversible or irreversible.

A similar profile of predicted effects could be generated for the substances listed in Table 3 and, once the predictions in terms of occurrence, onset, duration, and reversibility of effect are correctly predicted, then the criteria for test validation have been met. In addition, tests presumed to measure similar functions can be compared for the dosage level required to see an effect and for the capability to detect an effect where one is expected or show no effect where none is expected.

It is our belief that the acceptance of behavioral and neurological procedures for use in toxicology in general and neurotoxicology in particular will depend in large measure upon their demonstrated validity, sensitivity, and specificity. More importantly, validation of behavioral tests must occur before environmental agents with unknown potential for neurotoxicity can be assessed in any meaningful way.

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REFERENCES

1. Cabe, P.A. and H.A. Tilson. The hindlimb extensor response: A method for assessing motor dysfunction in rats. *Pharmac. Biochem. Behav.* **9:** 133–136, 1978.
2. Cabe,P. A., H. A. Tilson, C. L. Mitchell and R. Dennis. A simple recording grip strength device. *Pharmac. Biochem. Behav.* **8:** 101–102, 1978.
3. Casarett, L.J. Toxicologic Evaluation. In: *Toxicology: The Basic Science of Poisons*, edited by L. J. Casarett and J. Doull. New York: MacMillan, 1975, pp. 11–25.
4. Dews, P. B. Epistemology of screening for behavioral toxicity. *Envir. Hlth. Perspec.* **26:** 37–42, 1978.
5. Evans, H. L. and B. Weiss. Behavioral Toxicology. In: *Contemporary Research in Behavioral Pharmacology*, edited by D. E. Blackman and D. J. Sanger. New York and London: Plenum Press, 1978, pp. 449–487.
6. Goldstein, N. P., J. T. McCall and P. J. Dyck. Metal Neuropathy. In: *Peripheral Neuropathy*, edited by P. J. Dyck, P. K. Thomas and E. H. Lambert. Philadelphia: W.B. Saunders Co., 1975, pp. 1241–1262.
7. Luster, N., R. E. Faith and J. A. Moore. Effects of polybrominated biphenyls (PBB) on immune response in rodents. *Envir. Hlth. Perspec.* **23:** 227–232, 1978.
8. Miller, R. G. *Simultaneous Statistical Inference*. New York: McGraw-Hill, !966.
9. Moore, J. A., M. . Luster, B. N. Gupta and E. E. McConnell. Toxicological and immunological effects of a commercial polybrominated biphenyl mixture (Firemaster FF-1). Soc. of Toxicology, San Francisco, California, March 12–61, 1978, p. 153 (Abstract).
10. Moses, L. E. Quoted in: Goldstein, A., *Biostatistics, an Introductory Text*. New York: MacMillan, 1964, p. 62.
11. Norton, S. Toxicology of the Central Nervous System. In: *Toxicology: The Basic Science of Poisons*, edited by L. J. Casarett and J. Doull. New York: MacMillan, 1975, pp. 151–169.
12. Pitchumon, C.S., R. J. Stenger, W. S. Rosenthal and E. A. Johnson. Effects of 3,4-benzpyrene pretreatment on the hepatotoxicity of carbon tetrachloride in rats. *J. Pharmac. exp. Ther.* **181:** 227–233, 1972.
13. Principles for Evaluating Chemicals in the Environment. National Academy of Sciences, Washington, D. C., 1975.
14. Ringer, R. K. and D. Polin. The biological effects of polybrominated biphenyls in avian species. *Fedn. Proc.* **36:** 1894–1898, 1977.
15. Seppalainen, A. M. Peripheral nervous system in lead exposed workers. In: *Behavioral Toxicology*, edited by C. Xintaras, B. L. Johnson and I. deGroot. Cincinnati, Ohio: NIOSH, 1974, pp. 240–247.
16. Seppalainen, A. M. and M. Tolonen. Neurotoxicity of long-term exposure to carbon disulfide in the viscose rayon industry: A neurophysiological study. *J. Work Environ. Hlth.* **11:** 145–153, 1974.
17. Slight, S. D. and V. L. Sanger. Pathologic feature of polybrominated biphenyl toxicosis in the rat and guinea pig. *J. Am. Vet. Med. Assoc.* **169:** 1231–1235, 1976.
18. Spencer, P. S. and H. H. Schaumberg. A review of acrylamide neurotoxicity. Part I. Properties, uses and human exposure. *Can. J. Neurol. Sci.* **1:** 143–150, 1974.
19. Spencer, P. S. and H. H. Schaumberg. A review of acrylamide neurotoxicity. Part II. Experimental animal neurotoxicity and pathologic mechanisms. *Can. J. Neurol. Sci.* **1:** 152–169, 1974.
20. Szenozikowski, S., J. Stetkiewicz, T. Wronska-Nofer, and I. Zdrazkowska. Structural aspects of experimental carbon disulfide neuropathy. I. Development of neurohistological changes in chronically intoxicated rats. *Int. Arch. Arbeitsmed.* **31:** 135–149, 1973.
21. Tilson, H. A. and P. A. Cabe. Strategy for the assessment of neurobehavioral consequences of environmental factors. *Envir. Hlth Perpec.* **26:** 287–299, 1978.
22. Tilson, H. A. and P. A. Cabe. The effects of acrylamide given acutely or in repeated doses on fore- and hindlimb function of rats. *Toxicol. Appl. Pharmacol.* **47:** 355–362, 1979.
23. Tilson, H. A. and P. A. Cabe. Studies on the neurobehavioral effects of polybrominated biphenyls in rats. *Ann. N. Y. Acad. Sci.* in press.
24. Tilson, H. A., P. A. Cabe, E. H. Ellinwood, and L. P. Gonzalez. The effects of carbon disulfide on motor function and responsiveness to *d*-amphetamine in rats. *Neurobehav. Toxicol.* **1:** 57–63, 1979.
25. Tilson, H. A., P. A. Cabe and C. L. Mitchell. Behavioral and neurological toxicity of polybrominated biphenyls in rats and mice. *Env. Hlth. Perspec.* **23:** 257–263, 1978.
26. Tilson, H. A., P. S. Spencer and P. A. Cabe. Acrylamide neurotoxicity in rats: A correlated neurobehavioral and pathological study. *Neurotoxicology.* **1:** 89–104, 1979.
27. Vasilescu, C. Motor nerve conduction velocity and electromyogram in carbon disulphide poisoning. *Rev. Roum. Neurol.* **9:** 63–71, 1972.
28. Wang, G. H. Relation between spontaneous activity and oestrous cycle in the white rat. *Comp. Psychol. Monog.* **2:** 1–32, 1932.
29. Winer, B. J. *Statistical Principles in Experimental Design*. New York: McGraw-Hill, 1962.
30. Valciukas, J. A., R. Lilis, M. S. Wolff and H. A. Anderson. Comparative neurobehavioral study of a polybrominated biphenyl-exposed population in Michigan and a nonexposed group in Wisconsin. *Envir. Hlth. Perspec.* **23:** 199–210, 1978.

Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function
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Behavioral Epidemiology of Food Additives

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WEISS, B., C. COX, M. YOUNG, S. MARGEN AND J. H. WILLIAMS. *Behavioral epidemiology of food additives.* NEUROBEHAV. TOXICOL. 1: Suppl. 1, 149–155, 1979.—Behavioral toxicology in the natural environment can be considered a special branch of epidemiology. Behavioral epidemiology, because it typically relies on complex functional criteria, faces all of the problems of behavior measurement posed by uncontrollable variation, and amplified even further by chemical exposure. Many such issues arose in a study of behavioral responses to artificial food colors in children. Difficulties in employing Applied Behavioral Analysis in such a context run the gamut from selection of retrospective criteria to appropriate statistical models.

Behavioral epidemiology Food colors Applied Behavior Analysis Time series Food additives Randomization tests

WHAT happens to people is more compelling than what happens to rats. It also exerts more impact on public policy. Would a saccharin ban have been lifted by Congress had a few compulsive TAB drinkers testified to developing cancer? Moreover, we may not always be able to find a suitable animal model. High doses of amphetamine make humans act schizophrenic; they make rats gnaw. The natural environment, though, is such a thick web of frustration and uncertainty that it drives us to the epistemologic imperfection but methodologic comfort of the laboratory. Here, we will discuss the discomforts aroused by experiments in the natural environment and their implications for what we've called *Behavioral Epidemiology*.

Traditional epidemiology originated in infectious disease. Like traditional toxicology, death or pathology was its preferred endpoint. Morbidity data, such as hospital admissions, provide less satisfactory criteria because they are more subject to blurring by diagnostic variability and variations in seeking medical care. Functional measures, like those exemplified by behavior, magnify uncertainty. Aside from the tentative significance intrinsic to functional criteria, behavioral epidemiology faces, in exaggerated form, the threats to interpretation posed by transient effects, variable combinations of agents and exposure conditions, the staggering polymorphism of the human population, and wildly divergent behavioral histories. A sobering illustration of how such factors can combine into a puzzling montage is the debate over what constitutes safe exposure levels of lead for children. The population presumably most at risk has also inherited exacerbated risks from many sources.

The discipline known as Applied Behavior Analysis (ABA) offers a methodology for slipping some of these constraints, although at the cost of large samples. ABA grew out of attempts to transfer the technology of operant conditioning to the modification of human behavior. The technology could not be transferred wholesale. Elegant instrumentation

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[2] The material in this paper is based in part on a study conducted under FDA contract 223-76-2040, with the Kaiser Foundation Research Institute acting as primary contractor.

had to be left behind and total environmental control was out of the question. It retained the key element of precise specification of behavior, and developed recording schemes suitable for field use typically carried out by trained observers. As in animal studies, single subject data and interventions became the featured mode of investigation.

ABA shares common concerns with all behavioral approaches: stability, suitability, and sensitivity of baselines. As a component of toxicology and epidemiology, it must also incorporate an appreciation of dose or concentration as variables, the contribution of exposure duration and the impact on particular target organs. Its unique problems and opportunities arise from its ability to cope with several behavioral indices simultaneously, which implies the selection of several criterion behaviors, which poses the issue of single subject designs and the associated analytical constraints.

The potential, and the difficulties of applied behavior analysis as a tool of behavioral epidemiology can be illustrated by a recent experiment carried out in California. Its aim was to determine whether synthetic food colors can modify the behavior of susceptible children. It originated in the claim [3] that some of the children clinically labelled as hyperactive or hyperkinetic are actually exhibiting an elevated sensitivity to certain properties of synthetic food colors, flavors, and natural constituents chemically defined as salicylates. The data bearing on this central question will be published elsewhere. Here, we focus on the methodological issues that emerged in the design, execution, and analysis of the experiment.

Design Issues

Subjects and baseline selection. If acute effects are the primary question of a study, as they were here, a straightforward experimental design suggests itself. Select a group of allegedly responsive children whose diets already exclude the postulated offending substances and challenge them with one or more of these substances to determine if a reproducible reaction can be elicited. Such an approach obviously exploits a biased sample of children. It is most relevant when the validity of a phenomenon is at issue. It is a more basic question than prevalence.

Choice of criteria. The psychological literature overflows with behavior inventories, rating scales, and observational schemes. All have virtues and disadvantages. Inventories are useful in clinical assessment, as in helping a therapist specify the problem behaviors of a client. Most inventories, however, emphasize enduring behaviors or traits rather than transient states, making them unsuitable for assessing acute reactions. Rating scales, such as the Conners Parent-Teacher Questionnaire [2], which is widely employed to help diagnose hyperkinesis, provide a standard list of items, some of which may be relevant, some irrelevant to a particular subject. Standardization is an important virtue, however, and so is the feature that rating scale items typically integrate an observer's evaluation over a long time period (a day or longer). This allows the rater some flexibility in weighing specific incidents within their total environmental context. Their disadvantages are parallel. They usually do not reflect moment-to-moment variations in behavior. Often, the integration is secured at the cost of specificity in behavior, making the items subject to varying interpretations by observers, and, because of the long time spans often included, diluted by the same distortions as all retrospective surveys.

Actual frequency counts of narrowly defined behaviors at first seem an ideal solution. They are, certainly, for questions about the success of interventions. If, say, a child squirms excessively in the classroom, and the teacher is instructed to pay attention the child only when it is sitting still, the most direct criterion of effectiveness is the amount of squirming. Toxicology and pharmacology, however, because their interventions occur along nonbehavioral dimensions, may require a more comprehensive index of outcome. Typically, to the disappointment of those who have seen the productivity of observational methods in other settings, recording the frequency of specific behaviors has generally proven less sensitive to drugs than more global measures [6]. Narrow spectra of behaviors, combined with relatively brief (and unrepresentative) sampling periods may be at fault, but the empirical results are disappointing nevertheless.

Choosing observers. Highly-trained observers, thoroughly familiar with a specific coding scheme, represent an ideal that cannot be practiced, in large-scale studies, except at probably intolerable expense. The alternative is to select observers in situ, so to speak: teachers, nurses, parents. Because such observers are not trained specifically for such a role, and, further, often must fulfill responsibilities of a more demanding and immediate nature, they tend to produce more variable information than trained observers. But they have the advantage of unobtrusiveness, since they are part of the subjects' customary environment, and, also, familiarity with subtleties of the subjects' behavior not apparent to an outside observer. They also may be more biased, but can be provided with controls that minimize, or, at least, distribute bias.

Data analysis. Operant experimenters are accustomed to laboratory phenomena so robust that the data require little additional treatment. Such transparency is not characteristic of the natural environment. Important features may be buried in variability, in serial dependencies, in shifting baselines that cannot be extracted without some form of statistical surgery. These considerations became practical dilemmas when we set out to determine the validity of Feingold's assertions.

Specialists in Applied Behavior Analysis have been debating for several years the suitability of various statistical models and methods for data of this kind. Two sharp differences distinguish such data from the group designs typical of psychology. First, if each subject is assessed by a unique set of behaviors, and by a unique observer, grouping makes no sense. Second, conventional repeated measures models not only derive from group designs, but neglect the most critical feature of the paradigm: serial dependency. That is, by allowing several observations on the same experimental unit (subject), repeated measures designs do allow within-subject correlation to be modelled in the analysis. The experimental units, however, must be sampled independently. (This is true of any multivariate statistical procedure.) This requirement is not generally met when the same subject is observed at consecutive time points, unless some sort of washout occurs between observation. Such washout is the basis for the traditional crossover designs. Because of the nature of the challenge in this study, serial dependency from day to day clearly cannot be excluded. Therefore, any proposed statistical analysis must take account of (model) this dependency. Two methodologies are appropriate: time series analysis and randomization tests.

A *time series* is a sequence (in time) of observations (of a random process). The importance of this definition lies in its generality; statistical independence of the observations is not assumed, nor are the observations assumed to have any common probabilistic properties. Autocorrelations are important determinants of the behavior of a time series. The concept of simple correlation is familiar as an index of association between two variables (such as I.Q. and shoe size) measured on the same subject. If many observations (an entire time series) are taken on the same subject, then the first of them will be correlated with the second, and also with the third, fourth, etc. In many situations, autocorrelations decrease in magnitude as the lag or number of intervening observations increase, so that the influence of the past lessens with time.

There are two approaches to modelling the structure of the autocorrelations of a time series, corresponding to the parametric and nonparametric statistical models employed in group designs. The basic approach is to fit a parametric model to the data. The three most common stationary models are autoregressive processes, moving average processes and a combination of the two [5]. The first step in the analysis involves selection of one of the above models. This process involves the estimation and examination of the autocorrelations (which behave differently for each model) and some judgment, as well as any theoretical considerations which apply.

The nonparametric alternative is known as spectral analysis. This is basically an attempt to discover periodic behavior in the series caused by patterns in the autocorrelations. One basically tries to understand the behavior of the series in terms of the autocorrelations themselves, rather than specifying a model whose parameters determine the autocorrelations.

Randomization tests (like much of modern statistical methodology) originated with R. A. Fisher in the famous experiment of the lady testing tea [4]. (Chapter 2 of Fisher's classic reference still makes good reading.) The theory and practice of such tests has experienced a revived interest with the advent of third generation computers. The basic assumption made by all such tests is that the randomness necessary to generate the probability distribution of a test statistic is provided, not by random sampling of any sort, but by experimental randomization (the random assignment of experimental units to treatments). In the simplest case, if we have two groups, treatment and control, then we assign experimental units randomly to each of the two groups. If this is done, then, under the null hypothesis of no treatment effect, the particular arrangement of the observed data into the two groups may be considered simply a chance occurrence produced by the random assignment. We may test the null hypothesis by examining all possible rerandomizations of the observed data into the two groupings (treatment and control) of the same respective sizes (and category) and asking how unusual the originally observed differences are among all such permutations. As in any statistical test, unusualness is measured by a statistic. The p-value of the test is simply the percentage of rerandomizations whose statistic exceeds the value of the statistic for the observed data. The choice of the statistic is dictated by the alternative hypothesis of interest, i.e., a statistic is chosen which should be sensitive to the particular alternative.

As the numbers in each of the treatment groups grow, the number of rerandomizations quickly becomes enormous. Instead of calculating an exact p-value in such situations by generating all rerandomizations of the data, one can randomly sample from the set of all possible rerandomizations and estimate the true p-value. Monte Carlo studies have indicated that 10,000 is a suitably large sample.

The California Study

Experimental design. We decided to select only children successfully maintained (according to parents) on the diet, then challenge them with specific agents. Such an approach was feasible with synthetic colors, because only eight are approved for food by FDA, and one, Orange B, is restricted to sausage casings. There are 1500 synthetic flavors. We chose to administer a monoblend of the seven colors (excluding Orange B) in proportions and amounts based on a diet survey from which color intake was calculated by extrapolation from published manufacturing practices [1].

Over a 77-day experimental period, an individual child received eight color challenges. Every day, at a specified time, the child consumed a specially-formulated soft drink. On most days, it contained only a mix of caramel coloring and cranberry powder. On challenge days, randomly interspersed between Days 15–70, the drink contained the monoblend. The assigned occasions, however, sometimes had to be modified. Days on which the child consumed a forbidden item were eliminated from analyses.

Important variables lay beyond our control. We challenged with a dose equivalent to our calculated mean daily intake, although a more optimal design would have sought a dose-effect function. We were limited by the anxiety of human subjects review committees about the possibility of severe reactions. We administered the color blend on the average only once weekly because testimonial evidence suggested reactions to a single infraction lasting several days.

Specification of behaviors. Not only do standardized rating scales strike some observers as vague, but the most widely-used instruments for assessing hyperkinesis are based on children older than the ones in our sample. Our sample comprised 22 children, 15 boys and 7 girls, ranging in age from 2/5 to 6/11. All allegedly had responded successfully to the Feingold diet for at least three months before entry into the study. Since no single standard scale or inventory or coding scheme seemed suitable for all the children, we decided to develop a set of target behaviors for each child. The parent sorted a deck of 341 punched cards, each labelled with an item from a standard inventory. On successive sorts, the parent gradually narrowed the applicable items to 7 aversive and 3 positive behaviors. The aversive behaviors were to be those associated with infractions. Positive behaviors were included because it seemed possible that some reactions might be expressed more as a reduction in behaviors such as affection than as an eruption of behaviors such as aggression. Table 1 is a list of target behaviors from a representative subject.

We gauged the target behaviors in two ways. First, we had the parents count frequency of occurrence during two 15-min periods within the 24 hr period following the drink. One session took place within 3.5 hr of drink consumption, the other at a later time. These times were determined on the basis of parents' reports of when aversive behaviors seemed most frequent. Time of drink consumption and of observations remained uniform throughout the study period.

At the end of the 24-hr period (the *research day*), the parent recorded a global rating for each target behavior based on the total day. He or she marked a point on a line corresponding to "How much did it occur?" The position of

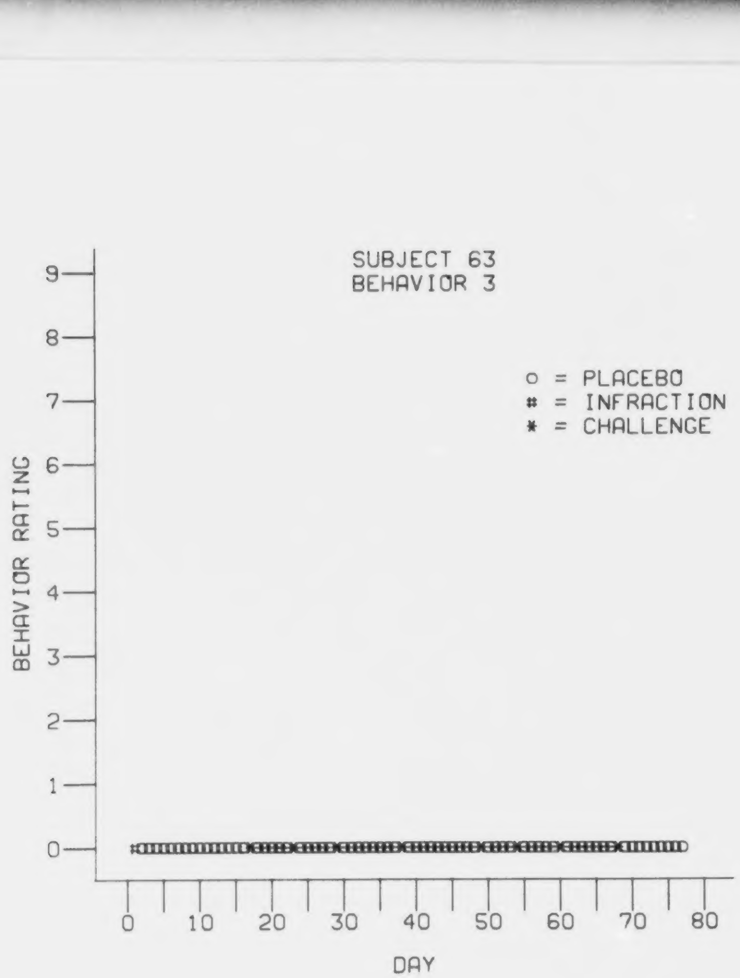

FIG. 1. Zero incidenceof a target behavior ("glazed eyes") selected as relevant by the mother.

TABLE 1

TARGET BEHAVIOR: SUBJECT 71

1. Short attention span, distracted
2. Fidgets
3. Mood changes drastically
4. Acts as if driven by motor
5. Will not eat enough
6. Loose bowels
7. Temper outburst
8. Responds to social stimulation by talking, smiling, etc.
9. Helpful, cooperative
10. Affectionate

the point was converted into a score ranging from 1–9. The parent also completed the Conners short form and supplied additional information and comments, including a total count of aversive behavior obtained from a wrist counter worn during part of the day.

Parents were telephoned every weekday to prompt them to complete and mail the forms, and to allow staff to conduct a partly-structured interview about the previous day's events. The interviewer and an independent listener then also completed corresponding forms. A behavioral specialist visited the home once weekly at a time during which the parent was scheduled to conduct one of the 15-min observations. She recorded the target behavior occurrences independently and compared her results with those of the parent. Such a procedure provides only a rough check of observer reliability, but at least it was able to uncover gross discrepancies in interpretation or a marked drift in criteria. These visits, along with weekly visits by the research nutritionist, served other functions as well. They helped maintain a warm personal relationship between staff and parents, a relationship crucial to a long, tedious, and demanding commitment by the parents. We emphasize this point because it is so often overlooked by experimenters who expect subjects to share their own interest and dedication without the rewards accruing to the experimenter.

Appropriate choices of behavior. We are all aware of dangers in the approach we adopted. One surely is reliance on retrospective ratings, a tactic that some questions impose because there are no alternatives, as in the studies of the Michigan residents exposed to PBBs. Recall that we asked parents to specify behaviors associated with diet infractions or that prevailed before adoption of the diet. All of the problems that accompany such approaches were amplified by the often startling rapidity with which children change their behavioral repertoire. Figure 1 is an example of a behavior selected by the parent as a characteristic aversive behavior. It never occurred during the course of the study.

Rapid change was another phenomenon we saw. Figure 2 plots a target behavior almost never recorded by the parent

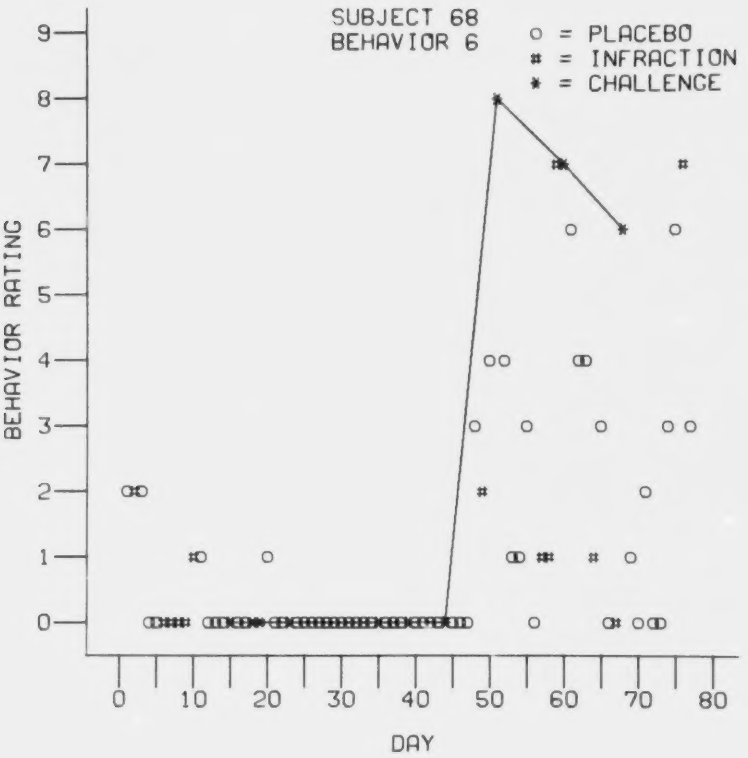

FIG. 2. Abrupt change in occurrence of a target behavior ("unable to stop a repetitive activity"). Challenge days are connected by a line.

during the first part of the study. It then suddenly appeared, and, in fact, demonstrated a statistically significant response to challenge ($p<0.01$). Did the parent's criterion change? Perhaps; but the other target behaviors did not reveal a similar pattern. Could the child have been "going through a phase?" Might the earlier challenges have exerted a cumulative impact?

One aspect of these data that surprised us, and that might prove helpful for behavioral epidemiology research, is the typically high correlations achieved among the global measures (total counts, day overall rating, and Conners score). For most subjects, these coefficients were about 0.70. Such a degree of concordance might suggest that, for some purposes, global rather than detailed measures are feasible indices of response or status. We must note, however, that without specified target behaviors, our observers might have been far less sensitive. Figure 3 (A and B) display daily ratings of two behaviors from one subject. Both behaviors were highly responsive to color challenge. Yet, their patterning during the study was markedly different.

Such peculiarities in patterning are partly what governed our choice of randomization tests as the most appropriate technique of data analysis. We simply could not meet the assumptions required by parametric approaches. Furthermore, we were dealing with phenomena that, even if most of the assumptions could be met, depart markedly from the usual designs in many respects. Although the parametric techniques employed in the first phase of the analysis did not lead us too far astray, they did, however, prove insensitive to subtle effects such as small elevations in the 15-min counts.

Outcome. The detailed results of this study will be reported elsewhere. For one child, the results were dramatic, even astonishing in their magnitude and consistency. Two or three other children showed significant reactions according to one or more target behaviors, so that they would not have been detected by global measures alone. The focus of this paper, however, is our approach, the problems it entailed, how they were handled, and the potential of this approach in the arena of environmental toxicology, because even those of us who strongly adhere to the operant tradition of intensive study of single subjects seemed baffled about how to transfer that tradition to toxicology. Perhaps we should stop worrying about screening and standard setting and return to the fundamental issue of what are the important variables.

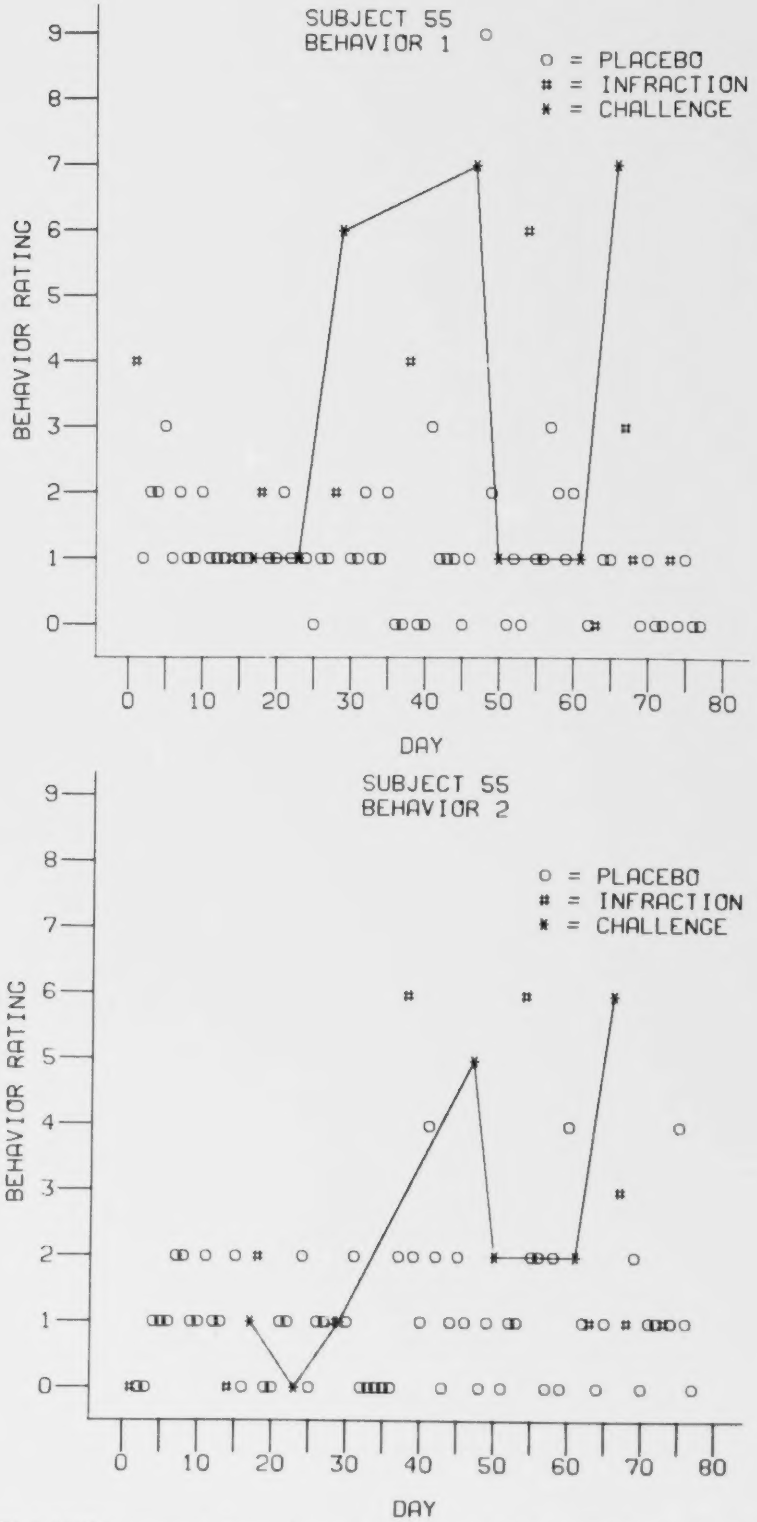

FIG. 3. (A) Response to challenge of target behavior, "Bites, kicks, hits." (B) Response to challenge of target behavior, "Throws things inappropriately." Lines connect challenge days.

REFERENCES

1. Certified Color Ir [illegible] Committee. Guidelines for food manufacturing practi [illegible] use of certified FD &C colors in food. *Food Technol.* **22:** 946–949, 1968.
2. Conners, C. K. Rating scales for use in drug studies with children. *Psychopharmac. Bull.: Pharmacotherapy of children.* Washington, DC: U.S. Government Printing Office, 1973, pp. 24–29.
3. Feingold, B. F. *Why Your Child is Hyperactive.* New York: Random House, 1975.
4. Fisher, R. A. *The Design of Experiments.* 5th edition. London: Oliver and Boyd, 1949.
5. Glass, G. V., V. L. Willson and J. M. Gottman. *Design and Analysis of Time-Series Experiments.* Boulder, Colo.: Colorado Associated University Press, 1975.
6. Werry, J. S. Measures in pediatric psychopharmacology. In: *Pediatric Psychopharmacology: The Use of Behavior-Modifying Drugs in Children,* edited by J. S. Werry. New York: Brunner/Mazel, 1978.

Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function
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Psychological Test Methods: Sensitivity to Long Term Chemical Exposure at Work

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HANNINEN, H. *Psychological test methods: Sensitivity to long term chemical exposure at work.* NEUROBEHAV. TOXICOL. **1:** Suppl. 1, 157–161, 1979.—Five studies dealing with long term occupational exposure to carbon disulfide, a mixture of organic solvents, toluene, styrene and lead are reviewed. All of the studies were cross-sectional, comprising either a comparison between exposed and nonexposed groups or the determination of exposure-response relationships, or both. The tests for the cognitive functions were known clinical intelligence and memory tests. The perceptual and psychomotor tasks were the Santa Ana test, the Bourdon-Wiersma test for visual-motor speed and accuracy, the Symmetry Drawing test and the Mira test. In four of the five studies the neurotoxic effect involved both cognitive and psychomotor functions. In the carbon disulfide group, psychomotor retardation was the most pronounced effect, in the group exposed to solvent mixtures the main effects were seen in the cognitive functions. The effects of styrene were limited to perceptual and psychomotor disturbances. As the most sensitive methods have varied from study to study, the continued use of broad and diverse psychological methodology in studies dealing with long term neurotoxic effects is proposed.

Environmental chemicals   Occupational exposure   Behavioral effects   Cognitive tests
Perceptual motor tests

THIS paper deals with the use of psychological test methods for determining central nervous system (CNS) dysfunctions in subjects with long term occupational exposure to toxic agents. Five empirical studies will be reviewed, and eleven of the test methods employed in these studies will be described.

Primarily the use of intelligence and memory tests will be dealt with, but some consideration will also be given to the perceptual and psychomotor tests.

All of the studies were cross-sectional, comprising either a comparison between exposed and nonexposed subjects or the determination of exposure-response relationships within the exposed group, or both.

METHOD

Table 1 gives a general view of the studies that provided the data for this paper.

In the carbon disulfide (CS_2) study the exposed group consisted of 50 exposed workers in a rayon fiber factory and 50 workers with manifest CS_2 poisoning. Fifty nonexposed workers from the same factory served as controls and they were matched with the exposed subjects in regard to age, length of employment and the type of job [4].

The subjects with exposure to solvent mixture were car painters from 27 repair shops in Helsinki. The mean exposure level corresponded to 32% of the Finnish threshold limit value for solvent mixtures. Locomotive assistants and engineers matched to the subjects according to age were used as controls [6].

In both studies the group differences in mean performances were evaluated with *t*-tests for the differences in separate variables, and by discriminant function analyses. In the car painter study the effect of possible differences in the initial intelligence levels was controlled by separate tests for group differences in a subsample where car painters were matched with respect to the preoccupational intelligence level; the test results obtained during military service were used in the matching.

The subjects with pure toluene exposure were photogravure printers. Age matched controls were picked out from the control group of the car painters [10].

Workers with styrene exposure were chosen from the reinforced polyester plastic industry and their controls were construction workers [9,11].

In each of the two latter mentioned studies analyses of exposure-response relationships were included in the data analyses. The individual toluene doses were evaluated on the basis of exposure histories obtained through interviews and noting the yearly consumption of toluene in the printing shops. Pertaining to the styrene investigation the mandelic acid concentrations in urine samples were used as a measure of the exposure level.

In order to investigate lead exposure, workers were chosen from two storage battery factories and one railway engineering workshop. The controls were nonexposed industrial workers. No individual matches were made but the group structure of the controls corresponded to that of the exposed groups in respect to age, sex, education and type of job [5]. The effects of low lead exposure were studied in a

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TABLE 1

OVERVIEW OF THE STUDIES

Exposure Agent	Number of Subjects	Age (years)		Duration of Exposure	
		Mean	Range	Mean	Range
Carbon disulphide	(50+) 50	38	25–50	11	5–20
Solvent mixture	100	36	21–60	15	1–36
Toluene	26	40	27–55	20	10–38
Styrene	98	30	17–55	5	0.1–10
Lead (total group)	88	36	22–58	8	2–28
Low exposure	49	33	22–53	6	2–20

subgroup containing only workers whose blood lead levels had been monitored during their entire exposure time and had never exceeded the level of 70 μg 100 ml. Emphasis in the data analyses was placed on the exposure-response relationship within the exposed group [7].

The Test Battery

A rather extensive test battery was used in all of the studies [8]. The rationale for using a large test battery was the fact that behavioral effects of chemicals must be considered by and large unpredictable as long as little is known about the main target sites and modes of action of various toxins in the CNS.

The structure of the test battery remained principally the same in all studies though the individual tests varied. All of the test batteries included cognitive tests as well as tests for perceptual and psychomotor functions. Table 2 presents the tests most frequently used.

THE COGNITIVE TESTS

The intelligence tests were subtasks of the Wechsler Adult Intelligence Scale (WAIS) [15], chosen because they are internationally used, based on general population norms and suitable for individual testing both with clinical and normal materials. Four subtasks of the Wechsler Memory Scale (WMS) [16] and Benton's well-known visual memory test [1] constituted the memory tests employed. They were chosen for the same reason as the intelligence tests.

Similarities

(Sim) is a test for verbal concept formation. It consists of 13 orally presented paired words and the subject has to find a similarity between the presented concepts.

Picture Completion

(PC) is a visual intelligence test that requires the subject to discover a missing part of an incompletely drawn picture.

Block Design

(BD) is a test for visual abstraction and has been widely used for detecting visual (spatial) disturbances in brain injury patients. The subject is given 4 or 9 two-colored blocks and for each of ten test items reproduces a two-colored design placed before him.

TABLE 2

THE TESTS USED TO MEASURE DIFFERENT PSYCHOLOGICAL FUNCTIONS

Test Category	Test
Intelligence	Similarities (Wais)
	Picture Completion (Wais)
	Block Design (Wais)
Memory	Digit Span (Wais and WMS)
	Logical Memory (WMS)
	Associative Learning (WMS)
	Visual Reproduction (WMS)
	Benton Test
Sensory and Motor Test	Bourdon-Wiersma
	Santa Ana
	Symmetry Drawing
	Mira Test

The Digit Span

The Digit Span (DSp) test is included in both the WAIS and WMS. It is more a memory test than a test of intelligence as the task is to recall digit series immediately after hearing.

The Logical Memory

The Logical Memory (LogM) test measures verbal memory to things logically associated together. A short story is read to the subject and he has to recall it.

Associative Learning

Associative Learning (Ass.L) consists of ten pairs of words to be learned during three trials. In each trial the word pairs are first read to the subject and then upon hearing the first word of each pair he must recall the second.

To determine visual memory either the Visual Reproduction (Vis.R) from the WMS or the Benton test were used. The Vis.R task contains three items, two of which have one figure to be remembered and one with two figures to remember. The Benton test contains ten items, eight of which contain three different figures to remember. The figures of the Benton test are simpler than those of the Vis.R task.

TABLE 3

SENSITIVITY OF THE COGNITIVE TESTS IN DIFFERENT STUDIES

Test	Exposure Agent				
	CS_2	Solvent Mixture	Toluene	Styrene	Lead
Similarities	—	+	+	—	(+)
Picture Completion	(+)	(+)	—	—	—
Block Design	+	+	—	—	+
Digit Span	(+)	+	—	—	+
Logical Memory	—	+	—	—	(+)
Associative Learning		+	+		
Visual Reproduction or Benton	—	+	+	—	+

— = no significant effect (+) = effect is slightly indicated + = effect is strongly indicated

When evaluating the effects as either slightly or strongly indicated, both the statistical significances of group differences and the support given by additional statistical treatment (discriminant function analysis, factor analysis, etc.) are taken into account. Exposure-dependent results are considered as stronger indications than the simple group comparisons. For more accurate information of the empirical results, see referents [4, 5, 6, 7, 9, 10, 11].

COGNITIVE TEST RESULTS

Table 3 gives an overview of the sensitivity of the intelligence and memory tests in the various studies conducted.

The car painters had the greatest number of statistically significant test results whereas the styrene workers had none.

The BD test which is generally considered to be sensitive to CNS dysfunctions was among the three best discriminators in the car painter study [6], and among the tests correlated with the blood lead levels in the lead study [7]. In contrast to this, PC and Sim are generally considered to be more resistant to CNS damages. Group differences in these particular tests must therefore be taken as warnings about possible preoccupational intelligence differences between the groups.

Concerning the car painter study this possibility was ruled out and deemed highly improbable in the toluene study. In the lead study, however, intelligence was a possible confounding factor in the group comparison, but not in respect to the relationship found between the uptake level and performances in the Block Design, Visual Reproduction and Digit Span tests.

The memory disturbances were pronounced in the car painter group, where all memory tests showed significant impairment and two of them—the Associative Learning and Digit Span tests—were among the three best discriminators [6]. In other groups the statistical significance of results obtained with the Digit Span test were at the 5% level (or else nothing at all). The significance of results received from the Logical Memory test followed these same trends as well. The Associative Learning test was superior to the Logical Memory test in the 2 studies where both of these tests were employed. The results received from the Visual Reproduction tests were in line with the oral tests.

THE PERCEPTUAL AND PSYCHOMOTOR TESTS

One cannot evaluate the utility of cognitive tests in the detection of toxic impairments without comparing their sensitivity to that of other kinds of tests. For that reason four tests measuring perceptual (visual) and psychomotor functions, or the coordination of them, will be briefly discussed.

The Santa Ana test is a visual-motor speed test. The equipment consists of a base plate with square depressions. Each depression contains an accurately fitting peg with a circular top. The task is to turn each peg in succession 180° as fast as possible. This test requires both eye-hand coordination and coordination of ths wrist and finger movements.

The Bourdon-Wiersma test measures visual-motor speed and accuracy. The test sheet has 50 rows each containing 25 groups of either three, four or five dots. The task is to draw a line through all of the groups of four dots as quickly and accurately as possible.

The Symmetry Drawing test is a method for detecting disturbances in visual perception and visual-motor coordination. The cognitive functions are more involved in this test than in the other perceptual motor tasks. Half of a figure of a tree leaf is printed on the test sheet and the task is to draw the other symmetric half. The number of reversions in the figure has been the most used variable in this task.

The Mira Test is used to measure psychomotor behavior. The task is to draw simple straight and broken lines without visual control, i.e., without seeing the paper and pencil. The subject sees the model at the beginning of each trial, but after the first lines are drawn a screen is put between him and the paper. The scores refer to the size of the drawing, to the deviations from the model line and to the disorganization of the movement pattern.

PERCEPTUAL AND PSYCHOMOTOR TEST RESULTS

Table 4 presents the results obtained by the perceptual and psychomotor tests. It can be seen that the psychomotor retardation was an essential part of ths CS_2 effects. They were clearly more pronounced in this group than were the cognitive impairments [4].

It can also be seen that the effects of styrene were limited to this behavioral domain: test variables measuring visual-motor accuracy and psychomotor performance were the

TABLE 4

SENSITIVITY OF THE PERCEPTUAL MOTOR TESTS IN DIFFERENT STUDIES

	Exposure Agent				
Test	CS_2	Solvent Mixture	Toluene	Styrene	Lead
Santa Ana	+	(+)	(+)	–	+
Bourdon-Wiersma, speed	+			–	–
Bourdon-Wiersma, accuracy	–			+	–
Symmetry Drawing	(+)		–	+	
Mira	+	(+)	+	+	

– = no significant effect (+) = effect is slightly indicated + = effect is strongly indicated

sensitive ones in respect to styrene exposure [9]. The effect was not a reduction in speed by a decrease in accuracy.

On the other hand the car painters with exposure to a mixture of organic solvents showed less pronounced impairments in the perceptual and psychomotor tasks than in the cognitive ones.

The Santa Ana test yielded significant results in four of the five studies. In two of the studies it was among the three best detectors [4,7]. The Mira test proved valuable in all studies where it was employed, though the best discriminating variable was not always the same.

DISCUSSION

The studies of long term effects due to occupational exposure to toxics always produce more or less soft data when compared with experimental studies made under laboratory conditions, because some compromises and approximations in the study design of field studies must always be made. If the group comparison method is used, then the critical factor is the matching of the controls to the exposed subjects. In principle the match should take into account all possible factors that can affect the measured behavioral variables, or at least the ones that are considered most essential. But in empirical studies one often has to be satisfied with an approximately similarity between the groups.

In determining exposure-response relationships the availability of reliable exposure data is the critical factor. Often, however, only the current exposures are known and the previous exposure levels and the total long term uptake must be an approximate evaluation.

It must be assumed that possible false approximations, poorly controlled confounding factors and intervening variables can, in these kinds of studies, produce noise that can hide or obscure the real information being searched for.

The confounding factors, such as age when correlated with the total exposure, or work loads connected with the exposure or its absence, can bias the results. The intervening variables, such as the different abilities and motivations of the subjects who may compensate for their functional impairments with increased effort, can mask the effect. The comparison of results that are obtained in different settings of subjects and by different study designs may help us to recognize the real information from the noise.

At the present there is some cumulative evidence from studies carried out in different countries about the usefulness of cognitive tests in the detection of the early effects of lead [2,14], carbon disulfide [12,13] and solvent mixtures [3] although the results differ in respect to the sensitivity of some of the individual tests. Special consideration of the sources of even minor discrepancies may be helpful in avoiding study design weaknesses which can produce either false negative or false positive results.

When discrepant results concern the sensitivity of certain behavioral domains to toxic effects, the discrepancy may be due to the different sensitivities of the tests used in different studies. Increased conformity between the methods used in the determination of toxic effects certainly would increase the cumulation of consistent information about these effects. This objective should not, however, discourage the efforts to enrich the methodology with new methods that have proven valuable in other domains of psychology.

The differing results in the studies reviewed in this paper cannot, except for a few minor points, be explained by methodological differences alone. Some of the differences seem to indicate real differences in the effects caused by different neurotoxic agents: the effects of styrene especially as well as those of CS_2 seem to differ from those of the other toxic agents. This finding points to different modes of action for these agents. Future research may throw more light onto this question.

Nevertheless, the comparison of results of several empirical studies reported here point to a relative unpredictability of the behavioral effects we are searching for. This also means that due to the present state of the available knowledge in this area it is not wise to reduce the test battery only to those methods that proved sensitive in some single study on some single exposure, but to proceed with a varied psychological methodology.

REFERENCES

1. Benton, A. L. The visual retention test: Clinical and experimental applications. New York, NY: The Psychological Corporation, 1972, p. 92.
2. Grandjean, P., E. Arnvik and J. Beckman. Psychological dysfunctions in lead-exposed workers: Relation to biological parameters of exposure. *Scand. J. work envir. Hlth* **4:** 275–283, 1978.

3. Hane, M., O. Axelson, J. Blume, C. Hogstedt, C. Sundell and B. Ydreborg. Psychological function changes among house-painters. *Scand. J. work envir. Hlth* **3:** 91–99, 1977.
4. Hänninen, H. Psychological picture of manifest and latent carbon disulfide poisoning. *Br. J. ind. Med.* **28:** 374–381, 1971.
5. Hänninen, H. Changes in performance, personality and subjective wellbeing as indicators of long-term exposure to toxic environments. In: *Proceedings of the 7th International Congress of Pharmacology*, 5, edited by C. Dumont. Paris: Pergamon Press, 1979.
6. Hänninen, H., L. Eskelinen, K. Husman and M. Nurminen. Behavioral effects of long-term exposure to a mixture of organic solvents. *Scand. J. work envir. Hlth* **2:** 240–255, 1976.
7. Hänninen, H., S. Hernberg, P. Mantere, R. Vesanto and M. Jalkanen. Psychological performance of subjects with low exposure to lead. *J. occup. Med.* **20:** 683–689, 1978.
8. Hänninen, H. and K. Lindström. *Behavioral Test Battery for Toxicopsychological Studies*. Used at the Institute of Occupational Health in Helsinki, 2nd revised edition. Helsinki: Institute of Occupational Health, 1979.
9. Härkönen, H., K. Lindström, A-M. Seppäläinen, S. Asp and S. Hernberg. Exposure-response relationship between styrene exposure and central nervous functions. *Scand. J. work envir. Hlth* **4:** 53–59, 1978.
10. Kalliokoski, P. *Toluene Exposure in Finnish Publication Photogravure Plants*. A thesis submitted to the faculty of the Graduate School of the University of Minnesota. Minnesota: University of Minnesota, 1978.
11. Lindström, K., H. Härkönen and S. Hernberg. Disturbances in psychological functions of workers occupationally exposed to styrene. *Scand. J. work envir. Hlth* **2:** 129–139, 1976.
12. Schneider, H. Möglichkeiten der Psychodiagnostik bei neurotoxischen Expositionen. In: *Adverse Effects of Environmental Chemicals and Psychotropic Drugs*, Vol. 2, edited by M. Horváth. Amsterdam: Elsevier, 1976, pp. 187–196.
13. Tuttle, T., G. D. Wood and C. Grether. *Behavioral and Neurological Evaluation of Workers Exposed to Carbon Disulphide*. Cincinnati, Ohio: U.S. Department of Health, Education and Welfare, 1976, p. 156.
14. Valciukas, J. A., R. Lilis, J. Eisinger, W. E. Blumberg, A. Fischbein and I. J. Selicoff. Behavioral indicators of lead neurotoxicity: Results of a clinical field study. *Int. arch. occup. envir. Hlth* **41:** 217–236, 1978.
15. Wechsler, D. *The Measurement and Appraisal of Adult Intelligence*. Baltimore: The Williams and Wilkins Co., 1958, p. 297.
16. Wechsler, D. A standardized memory scale for clinical use. *J. Psychol.* **19:** 87–95, 1945.

Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function
Neurobehavioral Toxicology, Vol. 1, Suppl. 1, pp. 163–174. ANKHO International Inc., 1979.

Quantitative Analysis of Rat Behavior Patterns in a Residential Maze[1]

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ELSNER, J., R. LOOSER AND G. ZBINDEN. *Quantitative analysis of rat behavior patterns in a residential maze.* NEUROBEHAV. TOXICOL. **1:** Suppl. 1, 163–174, 1979.—A method for monitoring spontaneous locomotor patterns of rats during one day is described. The animals' locomotion is registered in a residential maze by 18 optical gates connected to a computer. Status changes of each optical gate are stored on a disk file and can be retrieved for complete session reconstruction and data analysis. The general features of a rat's behavior in the maze are discussed. Quantitative analyses and statistical comparisons between two sessions spaced two weeks apart and between a group of 4 control animals and 4 rats treated in utero with methylmercury chloride are performed. Following parameters are analysed as functions of time and maze location: locomotor and local activity, occupational duration and time per visit in the maze compartments. Angular dependences of path decisions and regional preferences of crossings at the alley bifurcations are observed. No changes of the measured parameters can be observed between the first and second sessions. Methylmercury treatment results in a consistently lower local activity during the night period and in differences of path preferences.

Residential maze   Activity   Locomotion patterns   Behavioral toxicology   Methylmercury chloride

THE most critical stage in assessing the behavioral toxicity of new substance is the first one, when nothing or little is known about the effects on the behavior of living organisms. Behavioral toxicology traditionally uses psychopharmacological methods, which were developed to detect drug actions. There exists a great variety of different tests measuring very accurately specific symptoms resulting from interactions with receptors and transmitter mechanisms. Few methods have been described which give a first general idea about the spectrum of possible effects on the behavior.

Experiments in behavioral toxicology should fulfill two crucial requirements: the first is the stipulation that all types of behavioral effects can be recognized. The other is that non-specific disturbances of the nervous system, manifesting themselves in more generalized and diffuse subtle symptoms, should also be detected and measured. Since behavior is a very complex phenomenon that by itself cannot be defined and assessed, one may conclude that the best approach to behavioral toxicology would be to apply a battery of tests consisting of all known and established behavior assessment methods. Each test might then contribute to the overall information about the alteration of behavior. Such a procedure would be time-consuming and costly, and could thus only be applied to selected substances. A simple test procedure is therefore needed, which would give relevant clues about the types of behavior, if any, which may be altered by a chemical substance. Selected follow-up experiments may subsequently circumscribe more precisely the nature of the observed effects. This article explores the usefulness of a residential maze procedure as an initial and general screen for behavior alteration induced by chemical substances.

A residential maze is mainly a structured environment with a certain degree of complexity, in which the animal's behavioral interaction with its environment can be studied. No specific task is asked to be performed. But since the animal resides in the maze for a prolonged period of time, it is inevitable that certain behavioral patterns will develop, which are not likely to be random but structured and organized. The main task of the behavior analysis, therefore, does not simply consist in measuring activity, but must attempt to define the degrees of randomness and organisation of the behavior. In the process of defining the behavior, the animal itself is allowed to give the answer, and no predefined criteria will influence ideas about what "normal" behavior should be, and by how much these behaviors may vary. The only relevant criteria will be the stability within or across animals or animal groups.

One of the important advantages of such an experimental procedure is the ability to obtain a large amount of varied behavioral information from the animals in a toxicological experiment. At the same time the manipulations of the animals by the experimenter is minimal.

METHOD

The Residential Maze

The design of the residential maze is based on the work of Norton [4], who measured the activity of a group of 4 rats during

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[1] This work was supported in part by the Swiss National Science Foundation.

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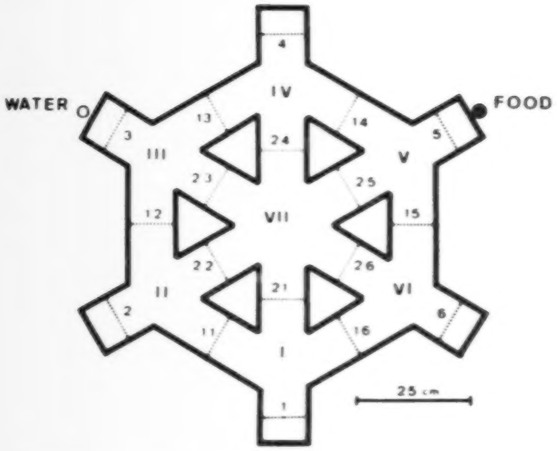

FIG. 1. The residential maze. The optical gate locations are indicated by dotted lines, numbered in groups of 6: the blind alleys (1–6), the circular alley segments (11–16) and the spoke alleys (21–26). Roman numbers refer to the alley crossings confined by optical gates. Blind alley 3 contains a water spout and 5 a wide wire mesh with access to food pellets. At the alley end of number 1, there is an opening at the top, measuring 2×9 cm. Its end-wall may be removed.

several days in an eight-shaped maze crossed by a straight alley, and on the work of Baettig [1,5], who studied the exploratory behavior of a single rat during 6 minute sessions in a rather complex maze structure. In our residential maze the activity patterns of only one rat at a time are observed for periods of 24 hours.

The maze configuration is a compromise between Norton's residential maze and Baettig's exploration maze (Fig. 1). It has a sixfold rotation axis of symmetry. Six concentric alleys emerge from the center and are connected near the periferal ends by a hexagonal alley. An infrared optical gate is located in each alley segment. It is connected to a digital computer through an interface system described elsewhere [2]. The alleys are 9 cm wide and 12 cm high.

The maze may be logically subdivided into 3 radial components containing 6 individual compartements each, numbered in clockwise order: (a) the blind alleys containing gates 1 through 6, (b) the circular alley segments with gates 11 through 16, and (c) the inner spoke alleys with gates 21 through 26. Likewise 6 circumferential compartements may be defined. They are numbered I through VI. A seventh compartement (VII) occupies the center of the maze and is confined by gates 21 through 26.

Three blind alleys are empty (gates 2, 4, and 6), the other contain a water spout (gate 3), a wide wire mesh letting access to food pellets (gate 5) and one has an opening at the top measuring 2×9 cm (gate 1). The front wall in alley number 1 can be removed for the introduction of the rat into the maze.

The maze is manufactured of 3 mm thick aluminum sheets screwed at the top to a 6 mm thick Plexiglas plate. This assembly is fixed on one end to a wooden plate by two hinges, allowing it to be lifted for removal of the rat and for cleaning at the end of the session. The floor is covered by a thin, easily removable hardplastic sheet, which is exchanged after each session for cleaning purposes. The maze can be illuminated for daylight simulation by two computer-switched lamps of 15 W each, located above gates 12 and 15. The maze can be rolled on two slides into a wooden box, providing about 20 dB of sound attenuation. The alluminum sheets defining the maze boundaries contain round holes 5 mm wide for ventilation. In addition, the wooden enclosure is ventilated by a fan.

Animals and Experimental Procedure

Two groups of 4 male ZUR:SIV-Z rats were used, each group belonging to one litter. One group (animals C1 through C4) was untreated. The dam of the other (aminals H1 through H4) was treated per os on days 6 through 9 of gestation (the day of conception being counted as day zero) with 2 mg/kg methylmercury chloride, a dose reported to have no effect on general motility levels and motor coordination of the pups as evaluated by swimming test, but impairing to some extent DRH performance at the age of 90 days [3]. Both groups were previously used for an ultrasound vocalization study, being isolated from their littermates for 1 minute per day on days 1 through 20 of their life, and on day 21 in a taste preference test (saccharine solution and tap water). Thus, the animals may be considered as naive with regard to the maze exposure, but they were handled daily in the first 3 weeks of their life.

The experiment started when the rats were 60 days old and lasted for 4 weeks. Each rat visited the maze twice for one day, the visits being spaced 2 weeks apart. A maze session started at 4 p.m. in the afternoon and lasted until 3:50 p.m. of the following day. The following schedule was used for both visits:

	Mon	Tue	Wed	Thur	Fri
1st Week	C1	C2	H1	H2	
2nd Week	C3	C4	H3	H4	

One week before and during the whole experimental procedure the animals were housed in individual cages. Weight gain and water and food consumption were monitored.

A rat was introduced at 4 p.m. into the illuminated maze and left undisturbed for 23 hr 50 min. At 7 p.m. the light was shut and lit again at 7 a.m. Every event as recorded by the optical gates was recorded by a PDP11/34 computer and stored on a disk file. Each record in the file contained the time of day in a resolution of 1/50 of a second, the gate number and its polartiy (break or release). The computer-controlled on and off switch of the light was also contained in the data stream.

The animal's weight before and after the maze session and its water and food consumption in the maze were measured. The defecation pattern was monitored by sketching onto a diagramm each defecation bolus as found on the floor after a day's residence in the maze.

RESULTS

Baseline Behavior in the Maze

A typical computer output of one day of a rat's locomotor activity in the residential maze is represented in Fig. 2. The 24 hours are divided into 12 strips of 2 hours each, labeled with the time of day. The optical gates are grouped in the

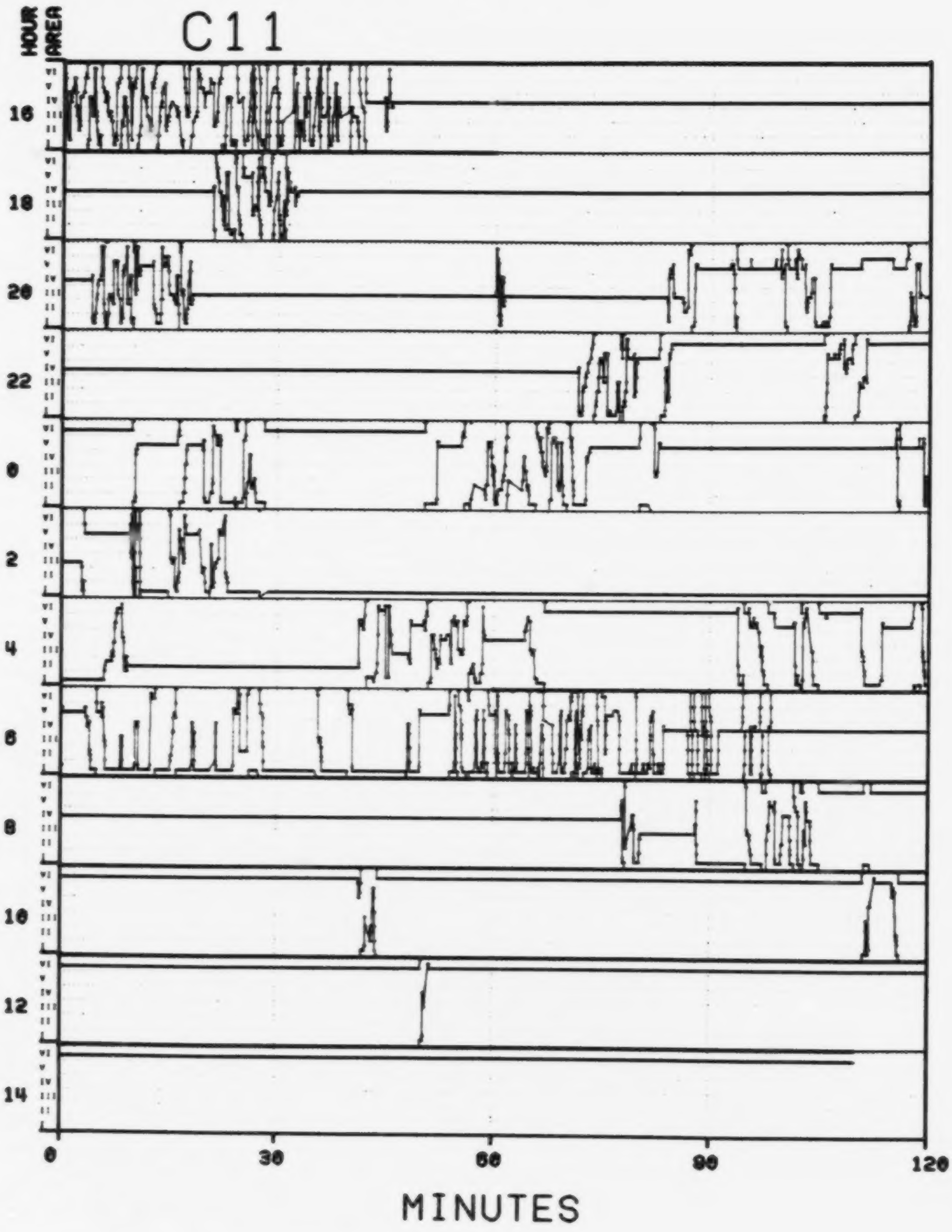

FIG. 2. Activity history of a one day session in the residential maze. The individual characters in the label C11 refer to the group (C), the animal number (1) and the session number (1). The time is represented in the horizontal axis in strips of 2 hours each. The optical gates are grouped in the vertical axis according to the maze regions I–VI defined in Fig. 1 (e.g. region I contains gates 21, 1, and 11, represented upwards in this order). Above each strip, a heavy line indicates that the light is on, and a thin line that it is off.

ordinate according to areas I through VI limited by the dotted lines. Within each gate group, the lower point corresponds to the blind alley, the higher to the spoke, and the one on the dotted line to the gate in the circular alley. The representation is circular in that the transition curves from region I to VI and from VI to I are not shown. Thin lines represent gate transitions and heavy lines visits in the gates. The straight line above each strip indicates the illumination status: a heavy line means the lights are on, and a thin line means they are off.

This representation allows a qualitative inspection of a rat's activity in detail. A rising curve showing visits in each region represents a clockwise walk through the circular alley, and an identical falling curve indicates a counterclockwise path. The crossing of one or more areas without any visits in them, indicates a crossing of the center square through the spoke of alleys. Backturns can be spotted through slope inversions.

A one day session can be somewhat artificially subdivided into four main periods: the exploration phase between 4 and 5 p.m., followed by the post-exploration phase from 5 to 7 p.m., the nocturnal phase from 7 p.m. to 7 a.m. and the diurnal phase from 7 a.m. to 3:50 p.m.

Besides showing a very high locomotor activity, the exploration phase is characterized by a high randomness of behavior. Every location in the maze is visited often during this time (in the order of 10 to 30 times each) and almost every path from one gate to another is walked through at least once. Also the timing of the activity reflecting the rat's walking speed is very irregular.

In the post-exploration period, the rat returns back to its diurnal rest, only occasionally interrupted by bursts of higher activity lasting up to 10 minutes. For resting the rat generally retires into a blind alley (most often blind alley number 4), the head turned towards the maze center.

The beginning of the nocturnal phase is almost indistinguishable from the post-exploration behavior. After 2 to 3 hours, a more or less sustained activity can be observed, showing repeated visits of blind alley 3 presumably for drinking and blind alley 5 for eating. A very high local activity (as defined by successive crossings of the same gate) can be observed in the food compartment, probably induced by eating activity. Also the blind alley 1 with the opening at the top is visited very frequently during this time, showing a high local activity as well. Before the switch-on of light, the activity is lower again, rising steeply right at the end of the dark period.

The begin of the diurnal phase is characterized by a period of very high activity, comparable to the one during exploration. Closer inspection of sequential features suggests a qualitatively different kind of behavior as compared to the exploration activity. It looks more orderly or even stereotype, containing for example rapid successive full circles around the circular alley.

The high activity early in the morning is followed by a very rapid decline of activity, resulting in a calm diurnal phase, which is only occasionally interrupted by short activity bursts. It is noteworthy that the choice of the sleeping compartement seems to be more or less definitive, as after short excursions the animal returns invariably back to the blind alley it has left.

Quantitative Analysis

In order to get a quantitative evaluation, the behavioral data in the maze have been analysed by several statistical procedures. The first analysis concerns locomotor activity defined as the number of gate crossings excluding repetitions of the same gate. The locomotor activity of the first session of animal C1 is represented in Fig. 3 as a function of the time of day and the gate number. The height of each cell in this three-dimensional histogram is proportional to the number of visits in a gate for one hour. The ordinate axis is scaled as percent of the total of visits in all gates and during the whole day. The background "walls" represent the sum of these values, the left wall showing the sum over all gates, and the back wall the sum over the whole day. The lines are drawn heavily, as long as the lights are on, and lightly when they are off.

The time evolution demonstrates quantitatively what has been discussed in the preceding section. The distribution over the gates shows that the rat spreads its activity rather evenly over the whole maze. This even distribution is modulated in a way which locates most locomotor activity in the circular alley (gates 11–16), and less in the spokes (gates 21–26) and blind alleys (gates 1–6). Focusing attention only

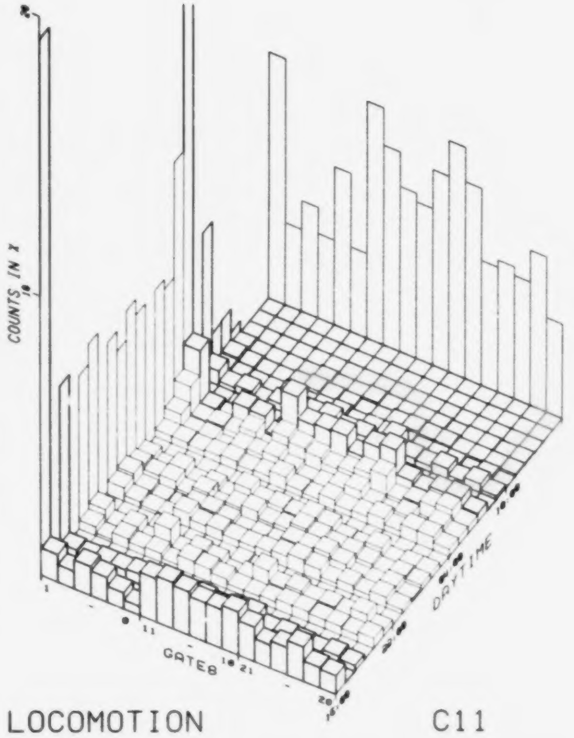

FIG. 3. Three-dimensional histogram representing the locomotor activity of C11 in function of time and gate number. The height of each cell is proportional to the number of visits in a gate for one hour. The gates are grouped into blind alleys (gates 1–6), circular alley segments (gates 11–16) and spoke alleys (gates 21–26). The vertical axis is scaled in proportion to the total visits in all gates and during the whole day. The background "walls" represent the sum of the values they are facing to. The lines are thick as long as the light is on, and thin, when it is off.

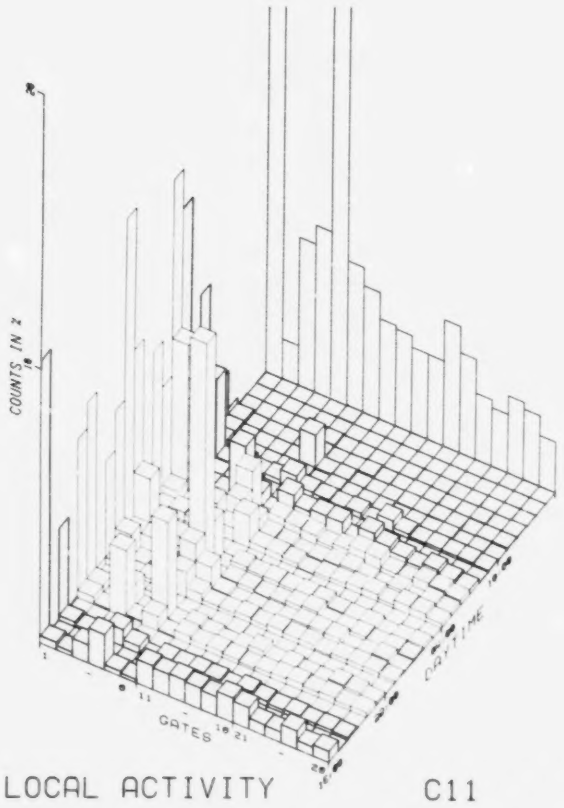

FIG. 4. Three-dimensional histogram representing the local activity (defined as the number of repetitive breaks and releases of the same optical gate) of C11 in function of time and gate number. For representational details refer to Fig. 3.

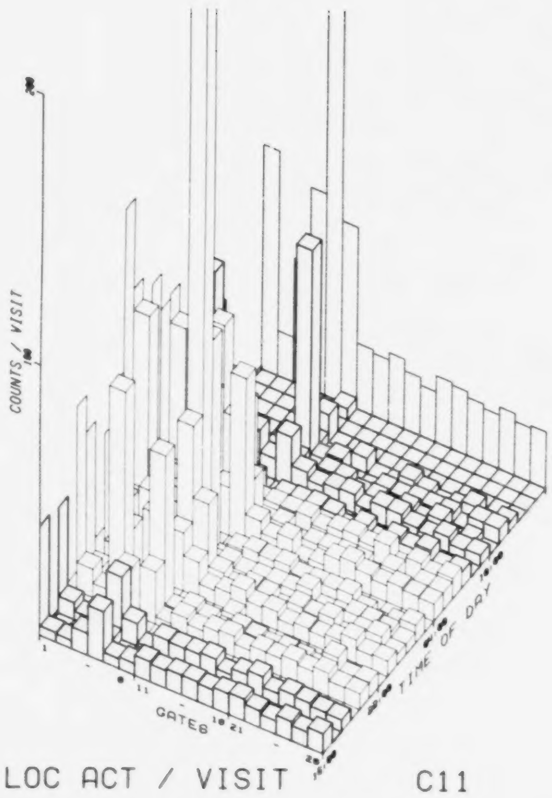

FIG. 5. Three-dimensional histogram representing the mean local counts per visit of C11 in function of time and gate number. This figure is calculated as the quotient of the values in Fig. 4 divided by those represented in Fig. 3. The mean values in the back "walls" are magnified six times in order to be seen. For representational details refer to Fig. 3.

onto the blind alley visits, the gate ! was visited markedly more often than the other five. Within the other blind alleys, food (gate 5) and water (gate 3) locations are next in frequency of visits. Differences within the other areas of the maze show also a concentration of the visiting rates around area I (gates 11, 16, and 21). In the topography of the temporal evolution of these visiting frequencies, one can observe that this preference becomes more pronounced in later stages of the experiment. This suggests that occupational habits are created during the maze session.

The next analysis focuses on the local activity, defined as successive breaks and releases of the same gate. Figure 4 is structured in the same way as Fig. 3. Compared to the locomotion, the local activity shows much more marked differences between the values in different location in the time-gate space. However, the local activity roughly parallels in time the locomotor activity. Additional high peaks of local activity during otherwise relatively calm night periods constitute the main difference between the two measures. These peaks are mainly due to feeding activity in the blind alley 5 and some "sniffing" activity in location 1.

The parallelism of the local and the locomotion activity counts is demonstrated in Fig. 5, which represents the number of local counts per visit as a function of time and maze location. Besides markedly different values in the blind alleys and random fluctuations over time, local activity counts per visit are constant.

The next analysis pertains to the time spent in the individual maze locations in proportion to the total time available (Fig. 6). The time spent outside a gate was evenly distributed between the areas defined by the last visited gate and the next one. The figure shows that the time spent in the areas of the circular (gates 11–16) and the spoke (gates 21–26) alleys is relatively constant and low. Most time is spent in the blind alleys (gates 1–6) during the whole session, excluding the exploration phase, when the occupational time is more evenly distributed among all maze areas. Thus, in contrast to the fact that the blind alleys are visited at about same or at even lower frequencies than the rest of the maze, these visits last much longer. This fact is represented in Fig. 7, where the mean time per visit is plotted, showing a large difference between the blind alley values (gates 1–6) and others.

The behavior pattern of single animals can be observed

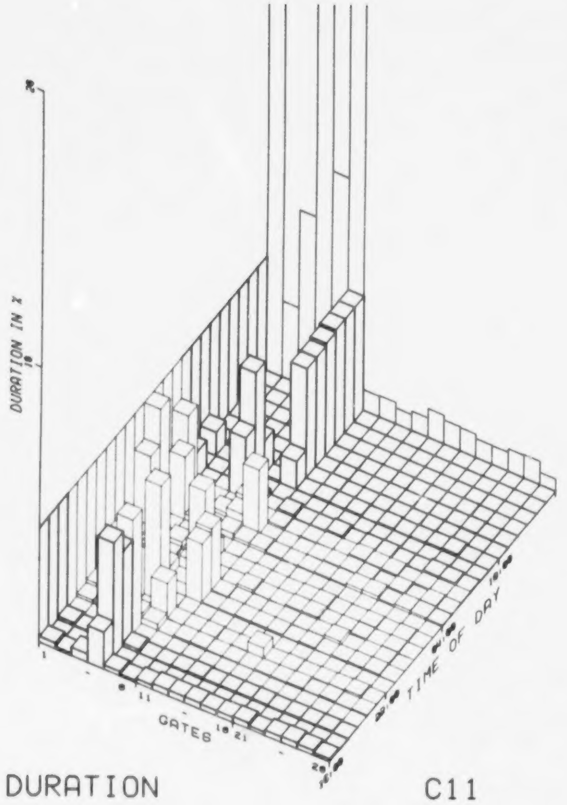

FIG. 6. Three-dimensional histogram representing the occupational duration in the maze compartments surrounding the gates, in proportion to the total time available. For representational details refer to Fig. 3.

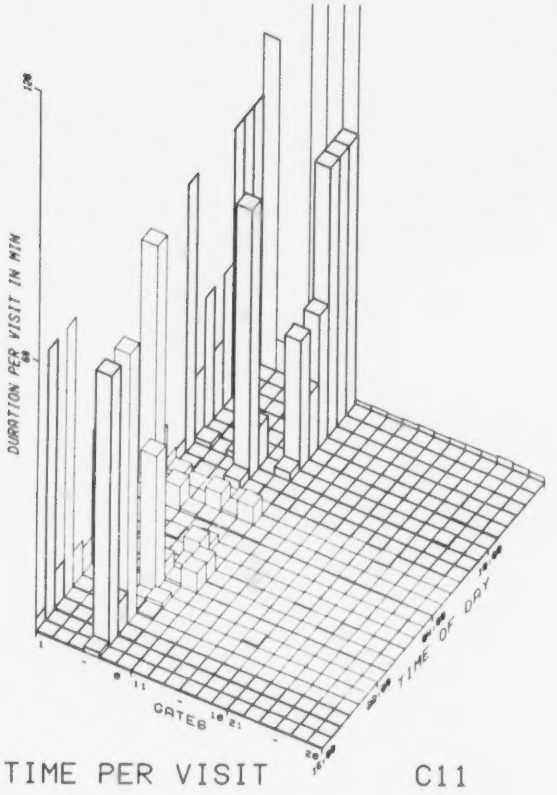

FIG 7. Three-dimensional histogram representing the mean time spent per visit in the compartments surrounding the optical gates. This figure is calculated as the quotient of the values in Fig. 6 divided by those represented in Fig. 3. For representational details refer to Fig 3.

also in pooled data of a group of animals. These values, besides having been smoothed to a certain extent by the pooling, show the same characteristics as those of single animals. Moreover, the behavior of rats in the maze exhibit a remarkable stability. This is illustrated in Figs. 8 and 9, where the time course of the mean locomotor and local activities of the same 4 animals are plotted, comparing the data of the first session with the second session two weeks later. The heavily drawn mean values are accompanied by lightly drawn confidence limits weighed in such a way, as to show *t*-test comparison results: no overlap of the two areas means a statistically significant difference at the 0.05 level. These plots demonstrate that even on a hourly basis and in considering separately the three main radial maze areas, the behavior in the maze is reproducible. This fact is confirmed also by paired *t*-tests made for all discussed parameters, in classifying the data into the three radial compartments and into the four main periods of the sessions. These tests result in fairly high probabilities of null hypothesis rejection error probabilities (all $p > 20\%$ and most $p > 50\%$).

The next analysis studies the frequency distributions of the time differences between two successive breaks and releases of alternative gates (locomotion) and of the same gate (local activity). Figure 10 shows that the frequency of time differences decreases with increasing intercount time. The shape of the curves demonstrate that they can not be explained by simple Poisson statistics, since the logarithmic transformation of the relative frequency distributions did not result in a straight line. However, the superposition of two or more exponential distributions would result in the observed shape. This fact indicates that the animal may be in two or more different distinct states of activity, each following a Poisson distribution with its own characteristic mean transition and repetition time. More detailed analysis is needed to study these aspects and to separate out distinct states of behavior.

The last study of the maze data deals with the relative frequencies of transition between the gates. Due to the maze geometry, not every transition is possible. Table 1 shows the matrix of transition counts in the first session of rat C1. In paranthesis are those observed during the first hour of exploration. Impossible and null transitions in the main diagonal are left empty.

The transition frequencies can be ordered in several man-

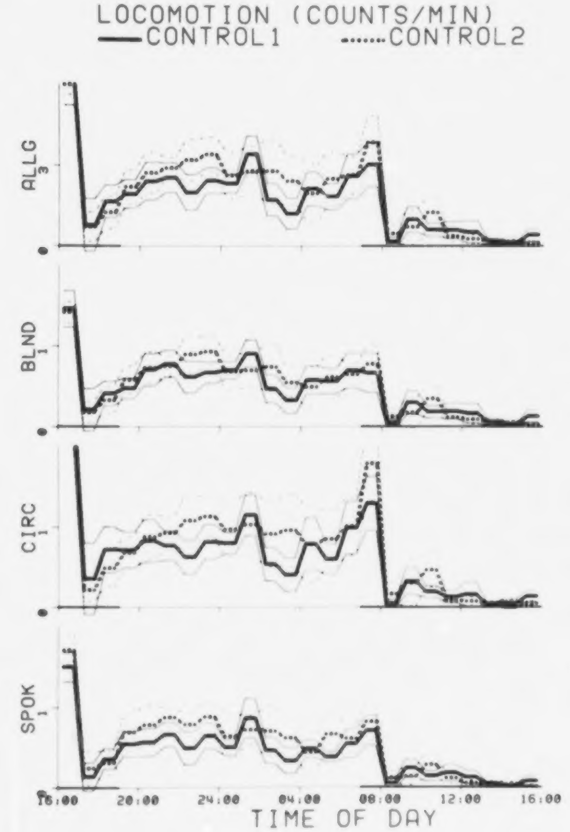

FIG. 8. Mean locomotion counts per minute (heavy lines) and confidence limits (thin lines) for two sessions spaced two weeks apart for the control group. The values for one hour, calculated as the mean of the 4 control rats is represented as plateaus in the curves. A heavy line on the time axis indicates that the light is on. The top figure shows the values of all gates, and the three lower ones the values of the three radial compartments (blind, circular and spoke alleys from top to bottom). Note the difference in scales between the top and other graphs.

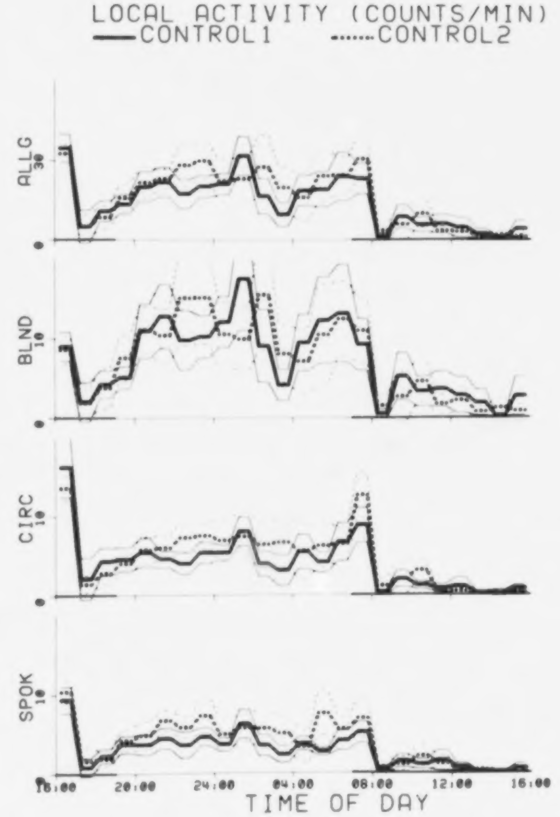

FIG. 9. Mean local activity counts per minute for two sessions spaced two weeks apart. For representational details refer to Fig. 8.

ners. Two ways of ordering are discussed here. The first, illustrated in Fig. 11, analyses the decision behavior at different types of bifurcations. The diagrams at the bottom of Fig. 11 have to be viewed as representing the decisions about which way to follow, if the animal is located in the bottom compartment and is proceeding upwards. The bar graphs above each diagram represent the corresponding decision frequencies, pooled over the six symmetrical situations in the maze. The top four hashed bar graphs are from the first sessions of the individual rats C1 through C4 and the black bar graphs at the bottom represent the pooled transition frequencies. Note the even distribution in the first case, the mirror symmetry between the second and third case and the remarkably symmetric distributions in the fourth and fifth case of the pooled data. These bar graphs indicate clearly an angular dependency of the bifurcation decisions, i.e. the obtuse angles are preferred. The only deviation of an angular function is seen in the first case. It may be explained by the fact that this situation results from a turn-around in the blind alley.

A second way of ordering the transition frequencies is according to the crossings of the seven squares I through VII and is represented in Fig. 12. In accordance with the locomotor activity, squares I, III, and V are preferentially crossed. The central square VII is crossed about as often or even less often than all the others, although there are more alleys leading to it. This finding may be explained by the observed angular dependency of bifurcation decisions.

Both orderings of the frequency distributions are relatively stable in comparing the first and second sessions of the control group. A chi square analysis of contingency tables results in high probabilities of rejection errors of the null hypothesis (all but one $p > 10\%$ and half of them $> 50\%$).

Two measures remain to be presented briefly: the defecation pattern in the maze as compiled at the session end is represented in Fig. 13. It demonstrates the defecations per area plotted against radial distances from the center. The accuracy of these data may be confounded by coprophagia. Nevertheless, a very consistent steep and monotonously falling curve as a function of the distance from the center results. Thus, although the center of the maze is less often

TABLE 1

C11 TRANSITION FREQUENCIES

	1	2	3	4	5	6	11	12	13	14	15	16	21	22	23	24	25	26
1							49 (3)					58 (7)	64 (9)					
2							17 (3)	22 (5)						19 (4)				
3								20 (5)	27 (10)						30 (7)			
4									18 (4)	12 (3)						29 (9)		
5										23 (4)	41 (2)						46 (5)	
6											19 (4)	26 (1)						13 (1)
11	63 (3)	9 (0)						43 (14)				22 (6)	13 (4)	15 (3)				
12		26 (8)	21 (6)				47 (3)		29 (8)					19 (3)	11 (4)			
13			25 (11)	13 (3)				35 (4)		24 (6)					16 (4)	6 (2)		
14				20 (3)	29 (4)				24 (7)		17 (5)					7 (3)	12 (1)	
15					44 (4)	11 (3)				29 (10)		28 (6)					13 (3)	7 (1)
16	46 (6)					23 (1)	28 (11)				19 (8)		12 (2)					13 (0)
21	61 (9)						12 (4)					18 (5)		2 (0)	9 (0)	20 (4)	13 (2)	2 (0)
22		22 (4)					17 (2)	16 (4)					3 (0)		1 (0)	10 (4)	15 (1)	5 (1)
23			11 (3)					37 (2)	4 (0)				10 (2)	2 (0)		3 (1)	10 (2)	20 (9)
24				22 (3)					13 (1)	10 (4)			22 (3)	8 (1)	8 (3)		6 (0)	3 (1)
25					37 (3)					31 (0)	16 (5)		14 (2)	21 (4)	7 (0)	1 (0)		1 (0)
26						15 (2)					8 (3)	9 (5)	1 (0)	16 (1)	12 (1)	10 (1)	1 (0)	

Frequency matrix of transitions between gates. The rows order the data according to the originating gates, and the columns according to the final gates. Impossible and null transitions (the main diagonal) are left blank. In parentheses are the values of the first hour only.

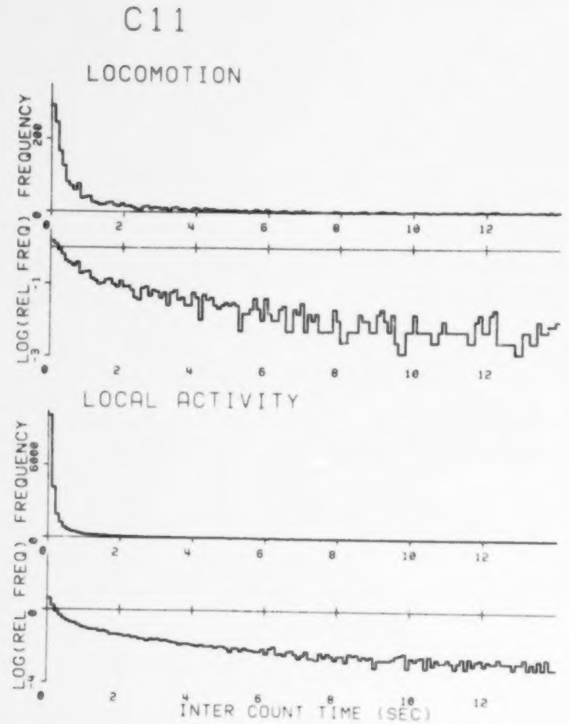

FIG. 10. Frequency distribution and logarithmically transformed relative frequency distributions of inter-count times for locomotor (top) and local activity counts (bottom), calculated from the whole one day session C11. The values are recorded at a resolution of 20 ms but represented at a resolution of 0.1 sec.

visited and the time spent in it is much lower than in other locations, it elicits the most defecations.

During the maze sessions the rats lose up to 20 g of weight, although food and water consumption is of the same order of magnitude as compared to home-cage data.

The Behavior of Methylmercury-Treated Rats in the Maze

Group comparisons of locomotor and local activities between control and treated groups of both maze sessions are shown in Figs. 14 and 15. The locomotor activity was not affected, while a consistent drop in the nocturnal local activity in the blind alleys and in the spokes can be observed. A two-factor analysis of variance was performed with the locomotor and local activities, with the occupational durations and with the time per visit values, one factor being the sessions and the other the treatment. No comparison resulted in a difference above chance level in either factor.

The transition frequencies of methylmercury treated animals are represented in Figs. 16 and 17. They differ significantly from the controls at both experimental sessions, as analysed by a chi square contingency table procedure: all but one of the 12 comparisons resulted in a $p < 5\%$, and half of them in a $p < 1\%$. This finding has to be confirmed by a repetition of the experiment.

DISCUSSION

The described experimental procedure for the assessment of psychotoxic effects on the behavior of rats was chosen mainly for the potentially high information content of the resulting data. To monitor all actions of an animal in the structured environment of the residential maze during a prolonged period of time, is an interesting method for behavioral research in itself. In order to be useful for toxicology, the measured parameters have to be stable and reproducible and at the same time highly sensitive to effects caused by chemical agents. It is also desirable that the resulting data are well interpretable with respect to underlying basic mechanisms of behavior, in order to lead the way to subsequent closer investigation of observed effects with more specific methods.

As far as stability and reproducibility are concerned, the study demonstrates that successive sessions of the same animal in the residential maze result in similar behavior, measured by locomotor and local activity, occupational durations in different maze compartments (absolute and per visit), and by several measures of compartment transition frequencies. This finding shows that the residential maze procedure permits longitudinal behavioral toxicology studies in which the animals can be used as their own controls.

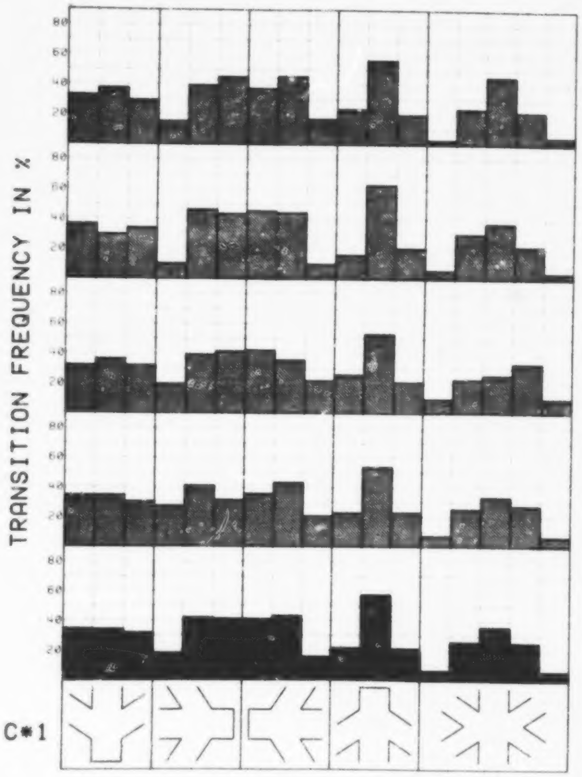

FIG. 11. Relative bifurcation frequencies of the four individual control rats (upper bar graphs) and for the pooled data (bottom) calculated from the whole one day sessions. The diagrams below the bar graphs indicate the represented decisions about which way to follow, if the animal is located at the bottom compartment and is proceeding upwards. The bar graphs are ordered according to the resulting path directions in the diagrams. The values are pooled over the six symmetrical situations in the maze.

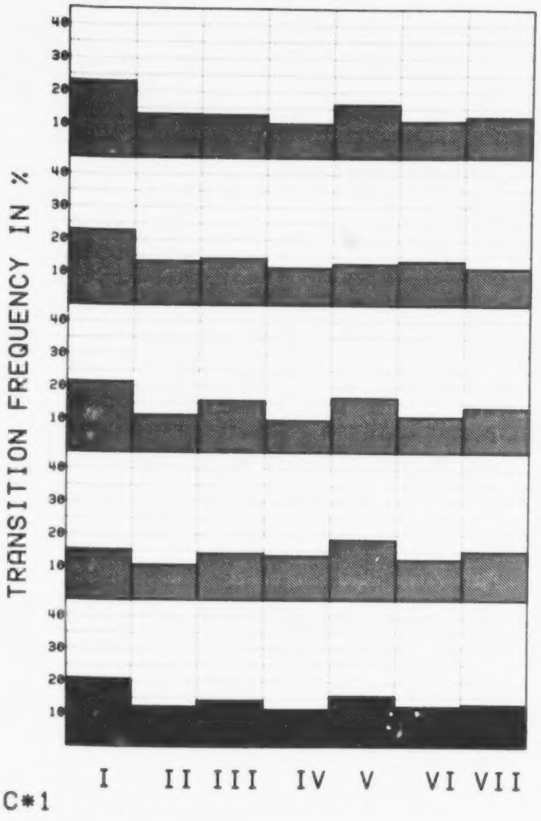

FIG. 12. Relative crossing frequencies of areas I through VII as defined in Fig. 1. The values are calculated over the whole one day sessions of the four control rats (upper bar graphs) and of the data pooled over the group (bottom).

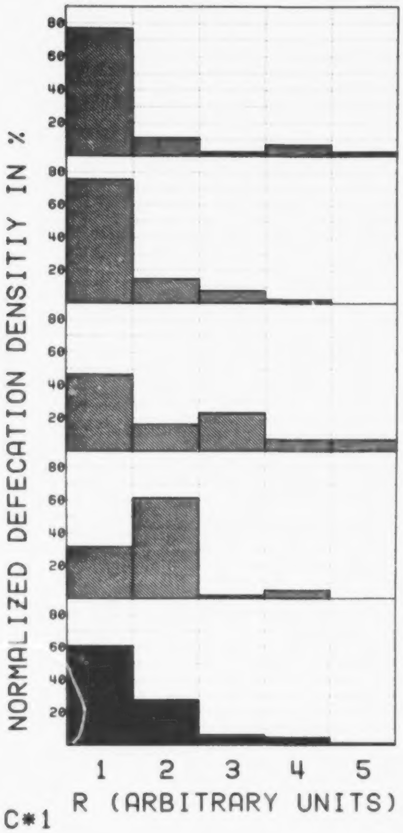

FIG. 13. Normalized frequency distribution of the number of defecation boli per area found on the floor at the end of the session for the four control rats. The radial distance from the center R takes following values: 1 for the center area, 2 for the inner spoke alley segments, 3 for the outer spoke alley segments, 4 for the circular alley and 5 for the blind alleys. Upper bar graphs represent the individual values and the lower graph the values pooled over the group.

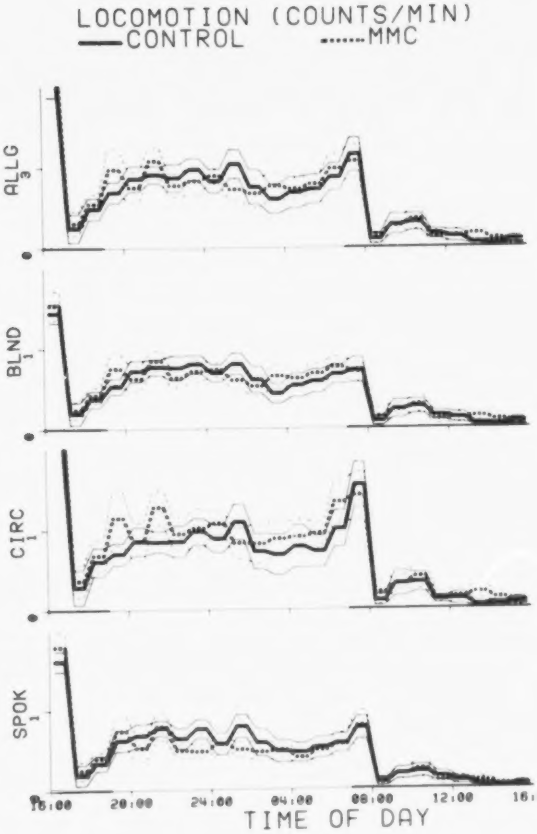

FIG. 14. Comparison between the mean locomotion counts per minute of the two sessions of the four control rats, and those of the methylmercury treated rats. For representional details refer to Fig. 8.

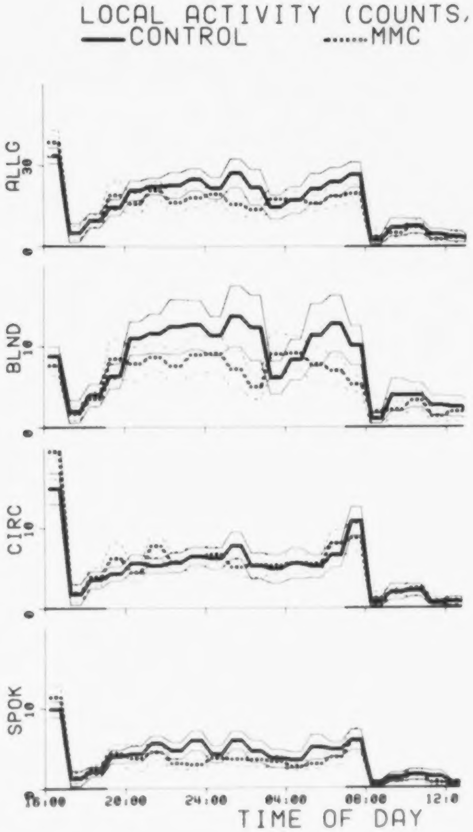

FIG. 15. Comparison between the mean local activity counts per minute of the two sessions of the 4 control rats, and those of the methylmercury treated rats. For representational details refer to Fig. 8.

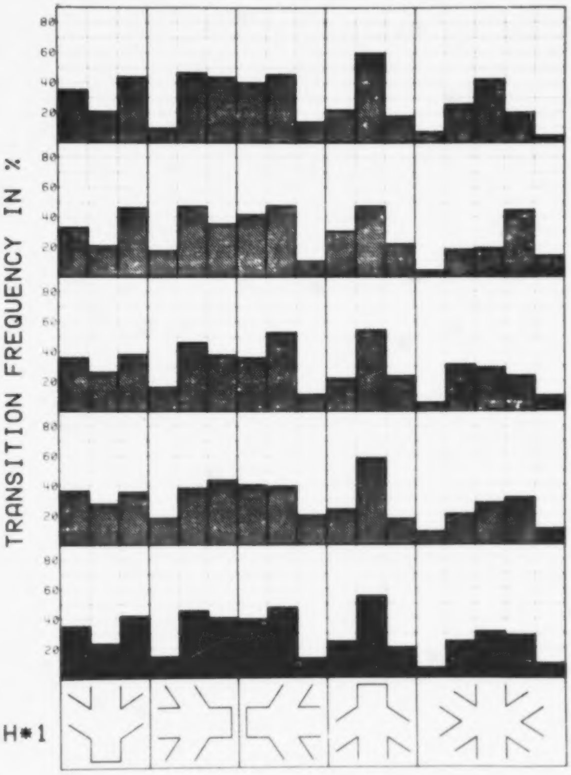

FIG. 16. Relative bifurcation frequencies of the four methylmercury treated rats. For representational details refer to Fig. 11.

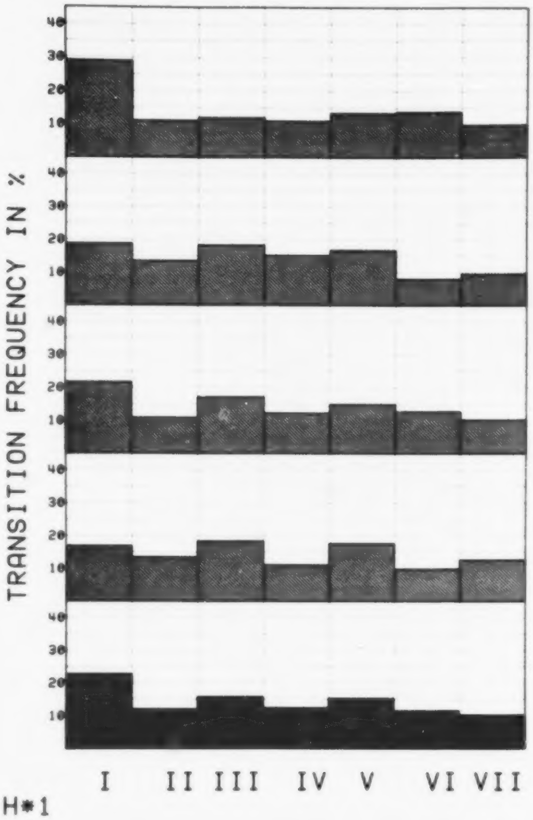

FIG. 17. Relative crossing frequencies of the four methylmercury treated rats. For representational details refer to Fig. 12.

REFERENCES

1. Baettig, K. and H. U. Wanner. Die spontane Lokomotion von Ratten in einer Kombination von Labyrinthgaengen und Offenfeld. *Helv. Physiol. Acta* **25:** 249–261, 1967.
2. Elsner, J. and R. Wehrli. Interface systems in behavioral research. *Behav. Res. Meth. Instrum.* **10:** 259–263, 1978.
3. Muesch, H. R., M. Bornhausen, H. Kriegel and H. Greim. Methylmercury chloride induces learning deficits in prenatally treated rats. *Archs Toxicol.* **40:** 103–108, 1978.
4. Norton, S., B. Culver and P. Mullenix. Development of nocturnal behavior in albino rats. *Behav. Biol.* **15:** 317–331, 1975.
5. Uster, H. J., K. Baettig and H. H. Naegeli. Effects of maze geometry and experience on exploratory behavior in the rat. *Anim. Learn. Behav.* **4:** 84–88, 1976.

Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function
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Comparison of Neurobehavioral Effects Induced by Various Experimental Models of Ataxia in the Rat[1,2]

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JOLICOEUR, F. B., D. B. RONDEAU, A. BARBEAU AND AND M. J. WAYNER. *Comparison of neurobehavioral effects induced by various experimental models of ataxia in the rat.* NEUROBEHAV. TOXICOL 1: Suppl. 1, 175–178, 1979.—The purpose of the present study was to design a standard battery of tests capable of quantitatively characterizing ataxia and concomitant neurological signs in the rat. In addition to a systematic analysis of the walking gait of animals, tests for activity, catalepsy, rigidity and various reflexive responses were included in the battery. The standardization of the test system was performed by determining and comparing neurobehavioral effects produced by 3-acetyl pyridine, acrylamide, pyrithiamine and thiamine deficiency, four experimental treatments reported to induce ataxia in animals. Results indicate that profiles of neurobehavioral disturbances accompanying ataxia in animals varied distinctively with each experimental treatment.

Ataxia 3-Acetyl pyridine Thiamine deficiency Pyrithiamine Neurobehavioral effects Neurobehavioral toxicology

ATAXIA frequently results from administration of neurotoxicants to experimental animals. However a standard method and procedure for detecting and measuring ataxia in experimental animals has not been developed. Usually, reports of ataxic symptoms in animals are based on qualitative observations. Possible concomitant neurological damage is rarely assessed. The purpose of the present study was to devise a standard battery of tests capable of quantitatively characterizing ataxia in the laboratory rat. The selection of the various tests was based on their proven ability to measure neurobehavioral changes induced by drugs or other manipulations in animals The standardization of the test battery was carried out by determining and comparing profiles of the neurobehavioral effects produced by 3-acetyl pyridine, acrylamide, thiamine deficiency and pyrithiamine, four treatments known to induce ataxia in animals.

A single injection of 3-acetyl pyridine in rats (75mg/kg) produces within 24 hours signs of cerebellar ataxia and damage to the medulla oblongata and climbing fibers of the cerebellum of rats [2]. Microscopic examination of the CNS reveals lesions as early as 7 hours after injection [3]. The ataxia resembles, both histologically and biochemically the olivocerebellar atrophy originally described by Holmes [7]. Recent studies have demonstrated that the lesions are associated with significant changes in the levels of certain animo acids in specific regions of the CNS [1].

Chronic administration of acrylamide in doses of 10–50 mg/kg in animals results in peripheral neuropathy characterized behaviorally by proprioception impairments, hindlimb paralysis, and progressive ataxia [6,10]. Histopathological examinations reveal distal axonal degeneration of peripheral motor and sensory nerve cells [16]. The neuropathy is first seen with a cumulative dose of 400 mg/kg and is most prominent with a cumulative dose of 500 mg/kg [4].

Chronic deficiency of vitamin B_1 results in pervasive metabolic and biochemical alterations in the nervous system. Thiamine deficiency induces a peripheral neuropathy of the "dying back" type which involves both sensory and motor nerve fibers [13]. When rats are chronically fed a thiamine-

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[1] The authors gratefully acknowledge the contribution of Dr. R. F. Butterworth, E. Hamel, F. Belanger and S. Gariepy. F.B.J. and D. B. R. were supported by the conseil de la Recherche en Santé du Quebec and the Medical Research Council of Canada respectively.

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free diet, a variety of neurobehavioral disturbances such as anorexia, piloerection, tremors, hypokinesia and ataxia develop at about 30–40 days from the start of the diet [12,19].

Chronic administration of pyrithiamine, an antimetabolite of thiamine, results in central histopathological changes mostly localized in the pons and medulla oblongata [19]. Pyrithiamine also causes axonal degeneration of peripheral nerves [19]. Manifestations of ataxic symptoms have been observed in animals injected daily for 18 days with 0.5 mg/kg pyrithiamine [5].

METHOD

Animals

Fifty four male Sprague Dawley rats, 275–350 g in weight, were used. They were divided into nine groups of six animals each. Food, which consisted of standard Purina Rat Chow, and water were available ad lib except when specified in the procedure.

Procedure

For 3-acetly pyridine, two groups of animals were used. One group received an acute intraperitoneal injection of 75 mg/kg 3-acetyl pyridine dissolved in physiological saline. Volumes of injection were 1 ml/kg. Animals in the control group were injected with an equal volume of saline. Animals were tested for ataxia and other neurological symptoms at 6, 12, 24, 48, and 72 hours following injection.

Two groups of animals were included in the acrylamide model. Animals in the experimental group received ten successive daily injections of 50 mg/kg acrylamide. Control animals were injected with saline. Acrylamide was dissolved in physiological saline and administered intraperitoneally in a volume of 1 ml/kg. Starting on the second day of acrylamide administration, neurobehavioral tests were carried out 30 minutes following the daily injection procedure.

For thiamine deficiency, one group of rats was given a thiamine-free diet (ICN Life Sciences, Nutritional Biochemical) throughout the experiment. Since this regime results in hypophagia with ensuing body weight losses, animals in the control group received standard rat chow in daily rations equivalent to the amounts consumed by the thiamine deficient animals. Neurobehavioral tests were performed on days 7, 14, 21, 27, 30 and 33; starting on day 35, tests were carried out daily until day 44.

To study the effects of pyrithiamine, three groups of animals were given the thiamine free diet and assigned to one of the following experimental treatments as described by Gubler [5]. Pyrithiamine treated rats were administered 100 μg/kg of thiamine and 0.5 mg/kg of pyrithiamine. Thiamine deficient animals received saline and control animals were injected with 100 μg/kg of thiamine. These treatments were given daily for 18 consecutive days. All substances were dissolved in saline and administered subcutaneously in volumes of 2 ml/kg. Animals were tested for neurological symptoms daily throughout the experiment.

The neurobehavioral effects induced by the various treatments were assessed by means of the following tests. The tests were performed in the order they are listed here. A more detailed description of testing procedures can be found in the original report of this study [9].

Locomotor activity. Spontaneous locomotor activity was measured for two minutes by means of a photocell activity apparatus (Lehigh Valley Electronics).

Catalepsy. Intensity of catalepsy was determined by placing the animal's front paws on a horizontal bar (1 cm in width) suspended 10 cm above the table. Time spent in that position, up to a maximum of 60 seconds, was recorded.

Rigidity. The rat was suspended by its front paws grasping a metal rod (0.5 cm diameter) which was held by the experimenter about 50 cm above the table. The time the animal remained on the bar (maximum 60 sec) was recorded. A prolonged grasping response has been correlated with direct measures of muscle rigidity [17].

Landing foot spread. After staining the hindpaws with ink, the animal was held horizontally 30 cm above a table covered with absorbent paper. The rat was dropped and the distance between the prints of each hindlimb was measured. This procedure has proved to be useful in detecting peripheral neuropathy in rats [11].

Gait analysis. After staining the hindfeet with ink, the animal was walked through an enclosed 90 cm long corridor with a paper covered floor. When two consecutive strides were obtained, the stride width, length and angle between consecutive steps on contralateral sides were calculated according to the procedure of Lee and Peters [11].

Reflexive responses. The presence of the righting and corneal reflexes as well as of a normal reaction to tail pinch was verified according to the procedure of Irwin [8]. The animal's ability to shift its weight during gravitational pull and the position of its hindlimbs when held vertically were then checked [15]. Subsequently, the presence or absence of a normal extension of the forelimbs when the animal, held by the tail, was lowered briskly toward a table top was also recorded [9]. Then, the animal, placed on a table, was lifted by the tail and the presence of a normal extension of the hindlimbs was noted [9]. Finally in the traction test, the animal was held by the tail and pulled horizontally on a table; the presence of a spontaneous hunched posture was recorded [9].

RESULTS

On each model, data obtained on activity, catalepsy, rigidity, landing foot spread and the three gait components were analysed by individual ANOVA's and the appropriate post hoc tests [18]. Results obtained on the various reflex tests were analysed by means of Fisher Exact Probability test [14]. In all cases, a difference between groups was considered significant if it had a probability of random occurrence of less than 5 percent.

For 3-acetyl pyridine, results obained at 6, 12, 24, 48 and 72 hours were included in the statistical analyses. It was found that 3-acetyl pyridine treated animals displayed more catalepsy and muscle rigidity, and had larger landing foot spreads than control animals at each of the five test periods. The treated animals were also found to be significantly less active than controls 6 hours after the injection but not at the other test periods. For the three components of gait analysis, no significant difference between the groups was found 6 hours after injection. However starting at 12 hours and for the remainder of the post injection test periods, treated animals were consistently ataxic as revealed by significantly smaller angles and stride lengths as well as by larger widths between steps. Analyses of the results obtained with the various reflex tests indicated the following significant effects. At 24, 48 and 72 hours, treated animals had lost the righting reflex and the ability to maintain a normal hunched posture during the traction test. Starting at 12 hours and enduring for the remainder of the experiment, treated animals displayed an abnormal hindlimb position characterized by the feet being retracted and held closely to the body. Finally a disturbance in the weight shift response of treated animals was found at the 72 hours post injection test period.

For acrylamide, data obtained in each of the nine daily test periods were included in the statistical analyses. In comparison to controls, acrylamide treated animals manifested significantly higher scores of catalepsy in all nine test periods. They also displayed significantly larger landing foot spreads than control animals in the fourth, sixth, seventh, eighth and ninth test period. Significant gait disturbances in acrylamide treated animals were found in all test periods including the first test period following acrylamide administration. The ataxia was characterized by significantly smaller stride angel and length and by larger widths between steps. The activity and rigidity scores of acrylamide treated animals did not differ significantly from those of control animals. Results on the various reflex tests revealed that in acrylamide treated animals the righting reflex was absent in the eighth and ninth test period and that the hindlimb extension response was impaired in the ninth test period. An abnormality in hindlimb position manifested by foot dropping and an inability to maintain a hunched position during the traction test were also found on the last test period. All other reflexes were unaffected by acrylamide.

For thiamine deficiency, results were analysed in two parts. First, a statistical analysis was performed on the data obtained on days 7, 14, 21, 27, and 33 of the experiment. This analysis indicated that only transient and sporadic effects were produced during this initial phase of thiamine deficiency. On day 21, thiamine deficient animals displayed significantly less locomotor activity than pair fed controls. A significant decrease in locomotion was also found on day 33 but not on day 27. The gait angle and width of deficient animals were respectively decreased and increased on day 27 while length of stride was unchanged. On day 33 stride length was significantly decreased in thiamine deficient rats but the other two gait parameters remained unaffected. The second part of the analysis dealt with the final phase of the experiment, i.e. days 35 to 44. During that phase, all thiamine deficient animals lost the righting reflex, displayed impaired weight shift responses and eventually died. These effects were not seen in pair fed controls. The time of occurrence of the neurological symptoms and of death in the thiamine deficient group varied from animal to animal. Because of this, and in order to uniformly compare groups, the results obtained on days when individual thiamine deficient animals lost their righting reflex were retained for analysis. Results collected in yoked pair fed controls on these days were also included in the analysis. No significant group differences were found for activity, catalepsy, rigidity and landing foot spread. Also, aside from the righting reflex and weight shift response, no other reflexes were affected in thiamine deficient animals. Finally, gait analysis revealed that in thiamine deficient rats the angle and length of strides were significantly smaller than those of pair fed controls.

Data obtained on days 1, 3, 6, 9, 12, 15, 16 and 17 of pyrithiamine administration were included in the statistical analysis. No significant group differences were detected for activity and rigidity. The landing foot spread of pyrithiamine treated rats was significantly larger than thiamine deficient animals throughout the experiment but did not differ significantly from controls. For catalepsy, the scores of pyrithiamine animals were significantly higher than controls on day 9. The catalepsy endured until the end of the experiment except for day 16 where differences between the two groups failed to reach statistical significance. Pyrithiamine had a minimal effect on the gait of treated animals. The width of steps in pyrithiamine treated animals was significantly larger than controls and this only difference did not occur before day 17 of pyrithiamine administration. Similarly, reflexes were not affected until day 17 when pyrithiamine animals lost their righting reflex and their ability to maintain a normal body posture during the traction test. Following the seventeenth injection the toxic effects of pyrithiamine precipitated. By day 18 two animals had died and the remaining rats were completely debilitated. In addition to the abnormalities found on day 17, these animals were incapable of sustaining locomotion, lost the forelimb extension reflex and could not emit a normal weight shift response.

An overall summary of the results obtained with all four models is presented in Table 1 where significant differences between treated animals and their respective controls are given for each treatment.

DISCUSSION

As expected, the four experimental treatments of this study induced ataxia as revealed by the gait analyses. However the overall pattern of neurological signs accompanying the uncoordinated gait varied distinctively from treatment to treatment.

Of all treatments, 3-acetyl pyridine produced the most diversified profile of neurobehavioral effects. This is not surprising in view of the known pervasive neurotoxic actions of this substance. A decrease in locomotor activity, an increase in landing foot spread, the presence of catalepsy as well as the appearance of a distinctive muscle rigidity were all apparent 6 hours after the administration of 3-acetyl pyridine which closely parallels the known time course of neuropathological changes induced by this substance [3]. However the first evidence of ataxia in this study was not found until the 12 hour test period following injection of 3-acetyl pyridine.

The neurobehavioral effects of acrylamide were different in several aspects. Ataxia and catalepsy were seen after the first injection of acrylamide while an increase in landing foot spread was not found until the fourth injection. The induction of a strong and persistent catalepsy by acrylamide was unexpected and it indicates that this substance might have widespread pharmacological effects in the CNS, aside from its documented neuropathological actions in the periphery. The rapid onset of ataxia after a single injection of 50 mg/kg is surprising in view of the known dose related neuropathological effects of acrylamide. As mentioned earlier, evidence of peripheral neuropathy is first detected after cumulative doses of 400 mg/kg [16]. This suggests that the early ataxic gait of acrylamide treated animals might appear before the neuropathological changes can be observed. The abnormalities observed in the righting, traction and forelimb extension reflexes as well as in the hindlimb position might be more directly related to neuropathy since they did not occur until the eighth injection, which corresponds to a cumulative dose of 400 mg/kg of acrylamide. In the hindlimb position test, acrylamide treated animals displayed foot dropping, an effect not seen with the other treatments of this study.

Contrary to expectations, thiamine deficiency and pyrithiamine did not yield similar profiles of neurobehavioral

TABLE 1

SUMMARY TABLE: OCCURRENCE OF NEUROLOGICAL SYMPTOMS IN ALL TREATMENTS

	Treatments			
	Thiamine Deficient	Pyrithiamine	Acrylamide	3-Acetyl Pyridine
Gait Analysis				
Length of steps	●↓		●↓	●↓
Width of steps	●↑	●↑	●↑	●↑
Angle of steps	●↓		●↓	●↓
General Signs				
Motor activity	●↓			●↓
Catalepsy		●	●	●
Rigidity				●
Landing foot spread			●↑	●↑
Reflexes (Loss of . . .)				
Righting reflex	●	●	●	●
Corneal reflex				
Traction		●	●	●
Forelimb extension		●		
Hindlimb extension	NT		●	NT
Hindlimb position			●	●
Weight shift	●	●		●
Tail pinch				

●Significant difference from control group ($p<0.05$)   ↑↓Direction of change   NT = not tested

effects. Only the righting reflex and weight shift response were affected similarly by both treatments. However all three components of gait were disturbed in thiamine deficient animals while only the width of stride was altered in pyrithiamine treated animals. Thiamine deficiency, unlike pyrithiamine, decreased locomotor activity. Catalepsy as well as abnormal traction and forelimb extension reflexes were observed with pyrithiamine but not with thiamine deficiency. These striking discrepancies in the effects indicate that thiamine deficiency and administration of pyrithiamine, an antimetabolite of thiamine, affect the nervous system by distinct biochemical and or neuropathological mechanisms.

Taken together, the results indicate that the battery of neurobehavioral tests used in this study constitutes a sensitive and reliable technique for detecting, quantifying and differentiating various ataxic syndromes in rats. The use of such standardized tests should prove to be valuable in studies of animal models of ataxia and in investigations of neurobehavioral effects induced by various neurotoxic substances.

REFERENCES

1. Butterworth, R. F., E. Hamel, F. Landreville and A. Barbeau. Cerebellar ataxia produced by 3-acetyl pyridine in rat. *Can. J. Neurol. Sci.* **5**: 131–133, 1978.
2. Desclin, J. C. Histological evidence supporting the inferior olive as the major source of cerebellar climbing fibers. *Brain Res.* **77**: 365–384, 1971.
3. Desclin, J. C. and J. Escubi. Effects of 3-acetyl pyridine on the central nervous system of the rat, as demonstrated by silver methods. *Brain Res.* **77**: 341–364, 1974.
4. Gipon, L., P. Schotman, F. G. I. Jennekins and W. H. Gispen. Description of the acrylamide syndrome in rats. *Neuropath. appl. Neurobiol.* **3**: 115–123,1977.
5. Gubler, C. J., B. L. Adams, B. Hammond, E. C. Yvan, S. M. Guo and M. Bennion. Effect of thiamine deprivation and thiamine antagonists on the level of gamma aminobutyric acid and on 2-oxoglutarate metabolism in the rat brain. *J. Neurochem.* **22**: 831–836, 1974.
6. Hamblin, D. O. The toxicity of acrylamide. A preliminary report. *S.E.D.E.S. Paris.* 195–199, 1956.
7. Holmes, G. A form of familial degeneration of cerebellum. *Brain.* **30**: 466–471, 1907.
8. Irwin, S. Comprehensive observational assessment. *Psychopharmacologia* **13**: 222–257, 1968.
9. Jolicoeur, F. B., D. B. Rondeau, E. Hamel, R. F. Butterworth and A. Barbeau. Measurement of ataxia and related neurological signs in the laboratory rat. *Can. J. Neurol. Sci.* **6**: 209–215, 1979.
10. Kuperman, A. S. Effects of acrylamide on the central nervous system of the cat. *J. Pharmac. exp. Ther.* **123**: 180–192, 1958.
11. Lee, C. C. and P. J. Peters. Neurotoxiciy and behavioral effects of thiamine in rats. *Envir. Hlth. Persp.* **17**: 34–43, 1976.
12. McCandless, D. S., S. Schenker and M. Cook. Encephalopathy of thiamine deficiency. *J. Clin. Invest.* **47**:2268–2289, 1968.
13. Schoental, R. and J. B. Cavanagh. Mechanisms involved in the 'Dying Back' process. *Neuropath. appl. Neurobiol.* **3**: 145–157, 1977.
14. Siegel, S. *Non-Parametric Statistics for the Behavioral Sciences.* New York: McGraw-Hill, 1956.
15. Snyder, D. R. and J. J. Braun. Dissociation between behavioral and physiological indices of organo-mercurial ingestion. *Toxic. appl. Pharmac.* **41**: 277–284, 1977.
16. Spencer, P. S. and H. H. Schaumberg. A review of acrylamide neurotoxicity Part II. *Can. J. Neurol. Sci.* **1**: 152–169, 1974.
17. Steg, G. Efferent muscle innervation and rigidity. *Acta Physiol. Scand.* **61**:Suppl. 225, 5–53, 1964.
18. Winer, B. J. *Statistical Principles in Experimental Design.* New York: McGraw-Hill, 1971.
19. Yoshimura, K., Y. Nishibe, Y. Inoue, S. Hirono, K. Toyoshima and T. Minesita. Animal experiments on thiamine avitaminosis and cerebral function. *J. Nutr. Sci. Vitaminol.* **22**: 429–437, 1976.

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Methodological Problems in the Analysis of Behavioral Tolerance in Toxicology

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BIGNAMI, G. *Methodological problems in the analysis of behavioral tolerance in toxicology.* NEUROBEHAV. TOXICOL. 1: Suppl. 1, 179–186, 1979.—The analysis of selected data on differential behavioral tolerance to drugs and other chemicals leads to a series of tentative methodological proposals with potential interest for the purposes of toxicology. These data show a wide range of different relations between tolerance induced by continued exposure to treatment per se and tolerance dependent on specific treatment-behavior interactions, such as behavioral testing in the treatment state and unfavorable consequences on reinforcement density of response changes induced by treatment. Consequently, when tolerance phenomena occurring with a particular type of treatment deserve an in-depth analysis, a sequential strategy should assess (i) critical factors in short-term compensation for behavioral deficits (acute behaviorally augmented tolerance), (ii) relations between sensitization and tolerance phenomena (particularly in the case of agents with long-lasting and/or cumulative physiological-biochemical effects), with special regard to tolerance development in the absence of measurable changes in the lower dosage ranges, and (iii) factors responsible for behaviorally augmented tolerance in medium- and long-term experiments. The latter analysis may require the investigation of different relations between time of treatment and time of testing, and different treatment-induced changes in reinforcement density. Specific and non-specific transfer of coping responses across situations must also be considered, as well as changes in response topographies, interindividual differences in rate of tolerance development as a function of size and direction of the original treatment changes, and several other cues which can facilitate the understanding of the phenomena observed. Several lines of work indicate that the search for separate types of mechanisms underlying different components of tolerance may have greater heuristic value than approaches based on continuum models of differential tolerance when attempts are made to single out critical physiological-biochemical mechanisms underlying various types of tolerance phenomena.

Behavioral tolerance Differential tolerance Behaviorally augmented tolerance Methods in behavioral toxicology Scopolamine Antimuscarinics Organophosphate anticholinesterases Amphetamine Stimulants Narcotic analgesics Cannabis derivatives CNS depressants (miscellaneous)

IN the year 63 B.C. the King of Pontus Mithridates (or, more correctly, Mithradates) the Sixth, repeatedly defeated by Roman forces led first by Lucullus and then by Pompey, and menaced by a mutiny of his own troops led by his son Pharnaces, attempted suicide by taking poison. A fatal intoxication did not follow: therefore, the king ordered a Gallic mercenary to slay him. This is how official history describes the death of Rome's greatest enemy in Asia Minor. The chronicle or legend, however, credits Mithridates with a life-long self-experimentation with increasing doses of various toxicants, carried out to create a protection against homicidal attempts. This story has been told here since it shows that an interest in tolerance has existed for thousands of years, and that experiments on desensitization by repeated exposure to noxious substances were started well before the beginning of the scientific age. In more recent times the attempts to analyze not only tolerance per se, but also the numerous irregularities observed in its development, have produced a great amount of data which have relevance at the descriptive, the explanatory, and the methodological levels.

The area covered by studies on tolerance is obviously too broad to be analyzed here in a systematic fashion. Several excellent reviews on behavioral tolerance are available (see especially [23,32]). The emphasis here will be on data and working models with special interest from a methodological viewpoint. In terms of the present state of knowledge it must be understood that a substantial portion of the relevant examples will be drawn from behavioral pharmacology, rather than from behavioral toxicology.

IMPLICATIONS OF SOME SHORT-TERM TOLERANCE PHENOMENA

It is well known [32] that behavioral changes during acute intoxication are often modified too quickly to allow a recourse to the more obvious explanations, such as those based on the chemical's disposition. In fact, many data on CNS depressants such as ethanol, hypnotic-sedatives, tranquilizers, narcotic analgesics, and cannabis derivatives (at least at relatively high doses) indicate that acute changes in sensitivity are the result of a treatment-behavior interaction triggering compensatory mechanisms which tend to reverse the behavioral imbalances produced by the treatments themselves (for example, impairment of postural regulation and motor coordination; reduction of consummatory responses; decrease in food or water reinforcement or increase in shock density as consequences of response changes in operant tasks).

Fairly simple tests can be carried out in order to assess whether or not a short-term modification of treatment effects can be ascribed to compensation due to treatment-behavior interactions. Consider, for example, the reduced response-reinforcement ratio often described in studies on cannabis derivatives using DRL tasks. During a test started 3 hours after treating monkeys with 0.75 mg/kg Δ^9-THC the animals can show considerable impairment in the first hour and remarkable (although incomplete) recovery in the second hour. Replication of the experiment with an interval of 4 hours between treatment and testing yielded identical results. This shows that short-term recovery is due to a factor other than passage of time from drug administration, and is caused by a mechanism other than waning of treatment consequences by drug metabolization (or by any other change taking place independently of the drug-behavior interaction) [39].

Another example can be drawn from studies on shuttle-box avoidance to assess dose-response curves in the case of neuroleptics. In fact, if a balanced cross-over-design is used, and tests are given to rats after increasing doses of chlorpromazine either with or without an interval of one hour between injection and testing, the response reduction is less if the animals are allowed to run continuously [31].

The methodological implications of these simple examples are self-evident, considering the large number of toxicants of different types which have depressant effects on the CNS. The suggestion is that dose-response curves over time should be assessed both within and between animals in order to separate attenuation and disappearance of effects due respectively to waning of the intoxication per se and to treatment-behavior interactions.

THE RELATIONS BETWEEN SENSITIZATION AND TOLERANCE

Empirical data have shown in several instances the existence of a *continuum* which includes (i) progressive increase in the size of treatment effects with repeated exposure, (ii) little or no change in sensitivity, and (iii) progressive tolerance. This has special relevance in the case of treatments with long-lasting effects on physiological-biochemical systems, as is the case with organophosphate compounds which inhibit cholinesterase. For example, an experiment comparing the effects on water intake of 0.2–1.0 mg/kg of DFP given intramuscularly on alternate days showed little deviation from the control level at the lowest dose, rapid deterioration and death at the highest dose, and a disturbance lasting several days, but ending in tolerance, at intermediate (0.4 and 0.6 mg/kg) dosage levels [18]. The 0.2 and 0.4 mg/kg groups differed in enzyme inhibition only so far as rate of reduction of enzyme activity was concerned, but not with respect to final levels. Furthermore, challenges by other treatments known to exert modified effects in tolerant animals (for example, increased sensitivity to the effects of scopolamine on drinking) indicated that tolerance was obtained both in the 0.4 mg/kg group and in the 0.2 mg/kg group, i.e., both in the presence and in the absence of a measurable disturbance during tolerance development. In other words, if a bias created by a treatment with cumulative effects is introduced very gradually, a profound change in the organism's physiological condition and behavioral reactivity can occur without any obvious sign of intoxication. The relevance of this phenomenon for toxicology is obvious.

The methodological implications of the aforementioned data and several others on organophosphates (for review and discussion see [7, 46, 48, 49]) is that compounds with long-lasting and cumulative effects on physiological-biochemical systems reflecting themselves in important behavioral changes should be tested by a battery of relatively simple methods (e.g., one or two activity tests, food and water intake, measurements of simple food- or water-reinforced operants, measurements of an avoidance response) in the course of continued exposure with different dosages. Treatments should be adjusted so as to determine the relations between (i) schedules causing progressive deterioration, (ii) schedules inducing deterioration, but leaving room for the development of tolerance, (iii) schedules possibly leading to tolerance in the absence of measurable changes, and (iv) a genuine no effect level. Obviously, in order to separate (iii) and (iv) appropriate challenges must be found for each type of treatment. Within a given category, however, there should be no difficulties of standardization, which makes it surprising that experiments such as that mentioned above on DFP and water intake [18] have not been replicated more widely in recent years.

Depending on the interest in a particular type of compound, and on the features of the agent to be tested, more and more specialized questions may have to be asked. For example, anti-anxiety agents do not induce a maximal disinhibition of responses suppressed by punishment during initial exposures. If an appropriate Geller-type schedule is used, with a high response rate in a component with intermittent positive reinforcement, and a very low response rate in another component with both positive reinforcement and punishment, the initial reduction of high rates often goes hand in hand with a modest increase of punished behavior. With repeated treatment the former change is attenuated, while the latter becomes more and more marked [11, 22, 41, 52]. Although some experiments show that the more obvious explanation (selective tolerance to the depressant component of the treatment action, leading to a progressive emergence of a disinhibiting component) may be too simplistic [22], one cannot overemphasize the relevance of these and related findings for behavioral toxicology. In fact, there are many situations in which shifts in the balance between response-enhancing and response-disinhibiting effects of a given treatment, interacting with those created by the contingencies which control behavior, may lead to unpredictable changes in the type of risk to which human subjects may be exposed.

Selective attenuation of response reduction, accompanied by maintenance (or even progressive increase) of response enhancements has often been described in the case of tests in which the former type of change has favorable, and the latter unfavorable, consequences at the reinforcement level (see later). The peculiarity of the shift obtained with anti-anxiety agents is that it takes place in the direction of a higher and higher punishment frequency via a more and more marked response disinhibition. The meaning of this phenomenon for the purposes of preclinical psychopharmacology and for the analysis of the mechanisms by which an anti-anxiety effect is obtained is outside the scope of the present discussion. There remains the fact that from a toxicological viewpoint a more accurate assessment of acute, subacute, and chronic effects of the drugs should be carried out, particularly with regard to weakening of response control by signals related to potentially harmful events.

THE ANALYSIS OF DIFFERENTIAL TOLERANCE

The representative data so far discussed suggest that in order to gain ample information on the behavioral effects of various treatments it is often necessary to analyze longitudinal changes in the profile of such effects as a function of different treatment-behavior interactions. This analysis can now be extended by making recourse to a somewhat unusual case, that of antimuscarinics (for more complete data and discussion see [2, 5, 6]). In fact, the illustration of data showing a complete separation of different types of tolerance can provide an appropriate contrast for a better understanding of ordinary cases which are characterized by a continuum of various types of tolerance phenomena.

The Unusual Case of Central Muscarinic Blockade: Complete Separation Between Different Types of Tolerance

If scopolamine is administered to rats prior to each training session on a FI schedule the characteristic response pattern induced by such schedules develops with considerable delay. Furthermore, this phenomenon is strictly related to slowly developing tolerance, since animals drugged after each of an extended series of training sessions exhibit a reduced treatment effect when eventually treated before sessions, relative to animals not drugged at all during training and then given a pre-test scopolamine challenge [16,17]. In other words, these analyses suggest a slowly developing tolerance on a metabolic basis or some other functional basis independent of any specified treatment-behavior interactions (e.g., receptor changes).

At the opposite extreme scopolamine effects on step-down avoidance are reduced in rats already at the second exposure, and post-test treatment does not allow such tolerance to develop [43,44]. Independent checks in the same laboratory on the effects of repeated treatment in a different situation (measurement of locomotor activity) did not show any tolerance [45]. In other words, the fast tolerance observed in the passive avoidance test appears to be due entirely to the treatment-behavior interaction. Prima facie evidence favors the hypothesis that animals stepping down from the platform in the drug state more frequently than in the control state and receiving many extra shocks can acquire an adequate coping response via substitute physiological-biochemical mechanisms not affected by the treatment.

In between the two extremes intermediate results are apparently obtained in some situations, for example, active-passive avoidance tasks which require crossing from one side to the other of a shuttle box during certain signals and response withholding during other signals. If appropriate stimulus combinations are used the initial effect of scopolamine (or any other centrally acting antimuscarinic) consists mainly of a passive avoidance disruption, and tolerance develops over a series of test sessions in the treatment state [1, 5, 15]. Also in this instance a comparison of pre- and postsession treatments showed that tolerance could not develop in the absence of a treatment-behavior interaction [15]. Additional checks on the role of reinforcement density were made with another go-no go task with similar features, except for the important fact that response suppression during the no-go stimulus complex was induced by extinction, instead of an avoidance contingency. The results indicated that in the absence of punishment for hyperresponding the maximal disinhibition produced by initial exposure is fully maintained in a series of sessions which would suffice to obtain tolerance in the face of a passive avoidance contingency [29].

Still other experiments contributed to the demonstration of the unusual features of differential tolerance developing in the presence of a scopolamine-induced alteration of reinforcement density. For example, in the active-passive avoidance task such tolerance does not consist of a shift to the right of the dose-response curve, since animals having regained response control in the course of a series of 1 mg/kg challenges were shown to be insensitive to doses up to 100 mg/kg [5].

Preliminary evidence in favor of a substitute-system explanation of behaviorally augmented tolerance to scopolamine was obtained by studying the interactions between drug treatment and frontal lesions which can induce a passive avoidance disruption similar to that induced by the drug. Animals made tolerant to scopolamine prior to lesioning showed a full fledged lesion effect, which simply confirmed that cortical areas are not critical for the induction of response changes by central muscarinic blockade. After the lesion deficit had also been compensated by retraining, however, sensitivity to scopolamine was again at a high level, while sham-operated animals showed maintenance of the drug tolerance acquired previously [4]. (This type of result, incidentally, provides little information on the nature of the mechanisms involved, but constitutes strong evidence for a role of systems not primarily involved in the drug-induced change in the development of behaviorally augmented tolerance.) Last, but not least, animals made fully tolerant to scopolamine in the go-no go task showed an EEG desynchronization indistinguishable from that observed after first exposure to the drug [28].

Similar indications have been obtained in still other situations. For example, if repeated scopolamine challenges are given to pigeons trained in a multiple FR–FI schedule one can observe two types of changes. The first, consisting of prolonged pauses which reduce reinforcement density, disappears within a few treatment sessions, while the second, consisting of an alteration of the FI response pattern, is maintained for an extended series of sessions [5]. Therefore, all the evidence suggests that in the case of central muscarinic blockade tolerance developing very slowly as a function of repeated treatment per se [16,17] and fast tolerance developing only in the face of unfavorable consequences at the reinforcement level are separated by a wide gap, and presumably require entirely separate neuropsychological and physiological-biochemical explanations.

Observational data have indicated that scopolamine-treated animals which have recovered response control, thereby meeting again reinforcement requirements, can behave differently from untreated animals. (Such differences in response topographies, obviously, would not be expected, if behaviorally augmented tolerance was served by the same mechanisms responsible for tolerance developing as a function of continued exposure per se (see [23]). Specifically, the hyperactivity and hyperreactivity induced by the drug was not modified by development of tolerance in the go-no go avoidance task. Furthermore, untreated rats performing at asymptotic level tended to remain quiet, or turned their heads around, or reared during presentation of passive avoidance signals. The same animals during scopolamine sessions, but after development of tolerance, often avoided self-inflicted punishment by taking brief runs towards the opposite side of the shuttle-box and then stopping just short of the midline [15]. A similar weeding of response components leading to noxious consequences, paralleled by a maintenance of other components of the drug-induced change, has been observed during active avoidance training with or without intertrial response punishment. In fact, active avoidance responses to the discrete signal were markedly enhanced by scopolamine in both conditions, while the tendency towards a higher intertrial activity was quickly suppressed only in the face of intertrial punishment [3].

The case of scopolamine has been illustrated in some detail not to suggest that experiments of a comparable complexity should be carried out systematically for toxicological purposes, but to facilitate the identification of methodological suggestions which do not necessarily require complex testing and evaluation procedures. Several important aspects of this process need to be discussed at this point, although it must be admitted that most inferences are quite tentative at the present state of knowledge.

Methodological Considerations

1. Initial steps in the search for differential (behaviorally augmented) tolerance. It is now widely accepted that the study of behavioral tolerance must assess the relative roles of treatment per se and of representative treatment-behavior interactions in bringing about a change in sensitivity. If the substance has a short metabolic cycle and does not induce a long-lasting change in physiological-biochemical systems some important preliminary information can be obtained simply by comparing the consequences of treatments administered respectively before and after behavioral tests. If, on the contrary, the effects of each exposure are prolonged, one can make recourse to tests yielding stable scores in spite of extended intervals between successive sessions (e.g., avoidance), and use a 2 × 2 design with treatment vs placebo as one factor and testing vs no testing as the other factor. In both instances animals treated after testing (or animals treated and not tested) must be treated before a subsequent testing session at a time when substantial tolerance has developed in animals tested in the treatment state.

Ad hoc variations of this strategy have allowed a separation of components of tolerance with quite different characteristics. For example, Δ^9-THC treatment prior to avoidance testing of rats leads quickly to a tolerance which is subsequently maintained for an extended period of time (even in the absence of renewed treatment and testing), while repeated dosing in the absence of testing leads to a tolerance which is subsequently lost in about two weeks [36]. Results of this type obviously suggest that it is necessary to search for separate (although often interacting) mechanisms, namely (i) changes in a chemical's disposition and/or changes in a chemical's effects on specific targets (e.g., drug-receptor interactions) taking place as a function of treatment per se, and (ii) shifts of response control from (a) target system(s) whose functioning is modified by treatment to (an)other system(s), as a consequence of treatment-behavior interactions triggered by changes in reinforcement density.

2. Further analysis of treatment-behavior interactions in differential tolerance. If the treatment studied is deemed to be worth an in-depth analysis one must proceed in an attempt to diversify the tests used, so as to obtain different combinations of treatment consequences at the response and the reinforcement levels. For example, efforts should be made not only to compare situations in which the treatment modifies, or conversely does not alter, reinforcement density, but also to compare situations in which a given change in reinforcement density depends on modifications of response rate in opposite directions (e.g., reduction of a high VI rate and increase of a low DRL rate; for the rationale, see later). Furthermore, an effort should be made to identify situations in which treatment-induced changes are in a favorable direction, as in the case of enhancements of response rate and reduction of shock rate in avoidance tasks (particularly if one uses a population of animals with a wide range of baseline avoidance performances). In fact, such changes are often maintained or even magnified with repeated exposure, providing a dramatic contrast with fast tolerance developing in the face of adverse treatment consequences at the reinforcement level (the discussion in [23] shows that this phenomenon is not limited to stimulants).

3. Checks on dose-response relations and carry-over of tolerance across tests. Independent confirmation of the relative roles of different components in tolerance can be obtained by analyzing dose-response relations before, during, and after tolerance development. In fact, if tolerance consists mainly of a shift of the dose-response curve to the right, then behaviorally augmented components—if any—should provisionally be viewed as if they were on a continuum with components obtained by repeated exposure to treatment per se. It would be incorrect in the absence of additional evidence to start systematic biochemical or physiological searches for separate tolerance mechanisms. Conversely, if behaviorally augmented tolerance generates an overall insensitivity over a wide range of doses—and this, of course, can be thoroughly verified only in the case of substances showing a wide ratio between toxic level and effective level—then one is authorized to think in terms of substitute mechanisms intervening in response control in the face of adverse consequences of a bias in the primarily affected system (see the scopolamine example).

Results obtained by dose-response studies can be confronted with analyses of carry-over of tolerance from one situation to the other. Quite obviously, carry-over of tolerance has a different meaning depending on whether or not the behaviors measured have a common learning element, which can be analyzed by conventional experiments on transfer of learning in the absence of treatment [38]. (For nonspecific carry-over of essential coping responses in the face of profound disturbances induced by treatment see a later section.)

4. Interpretation of tolerance in relation to type of initial change. All too often one forgets that an accurate analysis of the type of behavioral change produced by a given agent can help to understand the nature of treatment-behavior interactions in tolerance development. For example, if a substance has a general depressant and other incoordination effects, reflected in a drastic reduction of activity, impairment of balance, suppression of food and water intake, and impairment in a wide variety of operant and other learned behaviors, then homeostatic mechanisms are likely to be put to work to compensate for these deficits, even independently of any formal testing carried out by the experimenter. Alternatively, one can state that coping responses acquired by animals treated in their home environments and severely disturbed by the intoxication may transfer nonspecifically to a wide range of test situations [23].

Conversely, if the treatment consequences are more subtle, nonspecific transfer phenomena will play a lesser role, particularly in test situations in which reinforcement requirements are quite different from those of the home environments. In fact, as shown by the examples above and by the amphetamine data to be discussed later, in studies on tolerance to treatments which cause enhanced response rates and unfavorable changes in reinforcement density in DRL or passive avoidance paradigms conclusions have been in favor of a predominant role of the treatment-behavior interaction.

Although the real world is much more complex than suggested by any crude dichotomy, it is a fact that investigators working mainly with treatment effects of the former type have tended to support the view that tolerance induced by continued exposure per se and behaviorally augmented tolerance are strictly related to each other, and must be placed on the same continuum [7, 23, 32, 37, 38]. Conversely, investigators working mainly with effects of the latter type have rather emphasized qualitative differences between tolerance developing as a function of repeated treatment per se and tolerance due to specific treatment-behavior interactions [5, 6, 7, 23, 24, 25, 26, 27, 39, 40, 50, 51]. This controversy is more than a matter of semantics, since at some point it influences the choice between quite different strategies in the analysis of underlying mechanisms (see later).

Even setting aside those cases in which new coping responses have been observed during development of tolerance (see the scopolamine example illustrated in a previous section), an increasing number of tolerance phenomena are now being interpreted as depending on situation-specific interactions. For example, an apparently simple phenomenon such as behaviorally augmented tolerance to the analgesic action of morphine and related agents must be subdivided into different components, since at a behavioral level classically conditioned changes have been clearly separated from instrumental learning to cope with specified treatment consequences [30, 53, 54, 55, 56, 57]. Furthermore, it has recently been shown that development of tolerance to narcotic treatment can be prevented by the application of nociceptive stimulation concurrently with each drug experience [21]. This phenomenon, to be viewed in conjunction with absence of tolerance (or attenuated tolerance) to the discriminative stimulus properties of drugs [9, 19, 20, 47] and with the marked tolerance to the unconditioned stimulus properties of drugs [12, 13, 61], favors the hypothesis of an active regulation of the physiological consequences of treatment, with the biological significance of such consequences acting as the major factor in the regulation.

Any further discussion of these and other comparable phenomena would lead far away from the goals of the present analysis. Nevertheless, the warning must be that presently available criteria for the study and classification of tolerance phenomena may have to change quickly when adequate explanations are provided for those results which are still controversial.

5. Caveats on methods for the analysis of differential tolerance. The methodological criteria so far identified are all too easily overextended. In fact, a line of analysis which allows one to identify a major source of variance in the case of a particular treatment may well account for a much smaller portion of the variance in the case of other agents, or even prove to be completely useless under certain circumstances.

Presence vs absence of changes in reinforcement density have been repeatedly emphasized, therefore some data from the literature on amphetamine and related agents will be discussed to show the limits in the use of this discriminant. In fact, one finds both instances in which a reinforcement density model can account for most of the observed variation, and instances in which other factors must be responsible for differences in rate of tolerance development.

The original studies by Schuster and coworkers [50,51] and those carried out in recent years (see e.g. [10, 14, 26, 27, 58, 59]) have shown that consequences of response changes on reinforcement density have considerable importance both in the case of consummatory responses with a high survival value, and in that of responses trained with specified reinforcement contingencies (food- and water-reinforced operants, avoidance responses). For example, tolerance has been shown to develop to the disruptive drug effects on DRL responding, on response withholding in passive avoidance, and on response sequences, but not to modification of the FI response pattern, to facilitation of avoidance, nor to the effects observed in time-out periods.

As one turns to responses which do not belong to consummatory repertoires such as feeding and drinking, and have not been trained with specified reinforcement contingencies imposed by the experimenter, differential tolerance to amphetamine presents considerable difficulties of interpretation. For example, enhancements of locomotor activity and stereotypes tend to be maintained with repeated treatment, or even to become more and more pronounced (see, e.g. [8, 33, 35, 51, 59, 60, 62]. One should also note that measurements of activity have sometimes indicated an apparent reduction of the effect with repeated treatment: this phenomenon, however, is due to sensitization rather than to tolerance, since it goes hand in hand with a progressive increase of behavioral responses usually triggered by higher doses, such as some stereotypes [62]).

Other responses, on the contrary, show clear-cut tolerance, as exemplified by abatement of perseveration in a Y maze without a parallel reduction of overall activity [33,34]. Furthermore, it has been shown that such selective tolerance does not depend on testing in the treatment state, since it takes place also in animals treated after testing sessions [34]. In other words, either such abatement of the drug-induced perseveration is not behaviorally augmented, or a behaviorally augmented component must be ascribed to the development of coping responses opposing perseveration in the home environment, and to a subsequent transfer of such responses to the testing situation. Observational data fit to clarify this point are apparently not available.

It is obvious that by mentioning the difficulties in identifying sources of variance with certain types of treatments one scratches only the surface of a difficult problem. For example, in the case of amphetamine and related agents one should analyze at each step the treatment factors which might provide cues about biochemical substrates of differential tolerance, by considering the complex changes over time of different components of the neurochemical action of the drugs. The problem, however, is that a correlational (biochemistry-behavior) study might proceed at random if it is not supported by working hypotheses at a behavioral (neuropsychological) level which appear to account for a considerable portion of the observed variance.

6. Behavioral analyses versus studies on mechanisms underlying tolerance. Important advances in the analysis of tolerance (particularly differential tolerance) have been made in those areas in which working models at a behavioral (or neuropsychological) level have allowed identification of specific targets for physiological-biochemical analyses of underlying mechanisms. (A bolder statement paying homage to the late Robert McCleary might be that the further one moves away from considering behavioral responses simply as convenient dependent variables, the greater the probability that one will encounter substantial explanations [42].)

A satisfactory illustration of this point would require an extensive discussion of the studies on a specific class of compounds, for example, those on organophosphate agents which have permitted the establishment of a multifactorial model of tolerance development. In fact, a considerable portion of the observed behavioral variance can be ascribed to well-documented changes in muscarinic receptor sensitivity [48,49], and another portion to biochemical mechanisms which allow the return of acetylcholine towards normal levels in spite of a continuing enzyme depression, possibly by a selective fast recovery of some of the molecular forms of cholinesterase [7,46]. Furthermore, a third portion must tentatively be ascribed to a shift of response control from functionally impaired cholinergic neuronal systems to other systems in the face of the severe disturbances in the organism's homeostasis, since there is evidence for a behaviorally augmented component of tolerance which cannot be ascribed to the aforementioned mechanisms [7,46].

These and other advances with a similar meaning indicate that there can be at least heuristic value in postulating separate mechanisms for different components of behavioral tolerance: although—it should go without saying—what ultimately counts in the real world is the end product of higher order interactions between various mechanisms. Conversely, working models which tend to consider all differential tolerance phenomena simply as a series of different levels on the same continuum appear to have reduced heuristic value. In fact, either they lead to the search for a single type of mechanism, or they create difficulties in the search for different types of mechanisms by de-emphasizing those extreme situations in which one or the other of several interacting factors can be singled out.

This discussion should be terminated here, not only for reasons of space, but also to avoid confusion between a selective analysis of observed phenomena with direct methodological relevance and an extensive review of behavioral tolerance. For the sake of coherence, however, one more point must be made.

7. *The attitude of the experimenter.* If one looks back at the history of studies on tolerance it appears obvious that the identification of critical sources of variance and the proposals with high heuristic value for the analysis of underlying mechanisms have descended most of the time from a careful exploitation of apparent irregularities which could easily have been dismissed as disturbing variability. This applies, for example (i) to the finding of remarkable differences in tolerance development between animals showing behavioral changes in opposite directions upon initial exposure, and (ii) to the observation of different response topographies in instances in which automated recording yielded apparently identical performances.

This is not to affirm that standardized (run-of-the-mill) approaches in behavioral toxicology should be discarded altogether. In fact, once a particular methodological question has been solved for a given type of treatment, a considerable amount of routine work may be necessary in order to extend the analysis to a wider range of treatment schedules, to additional compounds of the same class, to different animal strains or species, and so forth. However, the investigator who runs a fully automated laboratory and never stops to think about differences between two individual records, or never takes the time to check whether or not similar response topographies correspond to a particular pattern in the records, might be overtaken by others more capable—as Rabelais might have said—to break the bone in order to suck the nourishing marrow.

CONCLUDING REMARKS

This discussion has to leave out two important extremes on a very wide continuum of problems. On the one hand, no attempt has been made to analyze results and explanatory models which are universally known, or methods which are sufficiently well established (this applies, for example, to the wide field of cross-tolerance analysis, although some asymmetrical results can create remarkable methodological problems [23]). On the other hand, only hints have been made to data and tentative models which are still too controversial to provide short- and medium-term methodological proposals for the purposes of toxicology.

The universe of behavioral tolerance phenomena with potential relevance for toxicology appears to be expanding rapidly. In fact, one sees today a fast development of new information such as that on classical conditioning phenomena in behavioral tolerance, on differential tolerance depending on the type of stimulus function exerted by a treatment in a particular context (e.g., no tolerance to a CS function vs tolerance to a US function at the same dose of the same agent), and more generally, on the many ways in which organisms can actively regulate the physiological consequences of treatment as a function of the changes in adaptiveness triggered by the treatment itself. A simple methodological solution to cope with these and other unavoidable complications is, unfortunately, not available.

REFERENCES

1. Bignami, G. Anticholinergic agents as tools in the investigation of behavioral phenomena. In: *Proceedings of the Vth International Congress of the Collegium Internationale Neuropsychopharmacologicum, Washington, D.C., 1966,* edited by H. Brill. Amsterdam: Excerpta Medica, I.C.S. 129, 1967, pp. 819–830.
2. Bignami, G. Nonassociative explanations of behavioral changes induced by central cholinergic drugs. *Acta Neurobiol. exp.* **36:** 5–90, 1976.
3. Bignami, G., L. Amorico, M. Frontali and N. Rosic. Central cholinergic blockade and two-way avoidance acquisition: The role of response disinhibition. *Physiol. Behav.* **7:** 461–470, 1971.
4. Bignami, G., G. Carro-Ciampi and M. Albert. Effects of frontal lesions on "go-no go" avoidance behaviour in normal and scopolamine-treated rats. *Physiol. Behav.* **3:** 487–493, 1968.
5. Bignami, G. and G. L. Gatti. Repeated administration of central anticholinergics: Classical tolerance phenomena versus behavioural adjustments to compensate for drug-induced deficits. In: *Sensitization to Drugs, Proceedings of the European Society for the Study of Drug Toxicity, Xth Meeting, Oxford, 1968,* edited by S. B. de C. Baker and J. Tripod. Amsterdam: Excerpta Medica, I.C.S. 181, 1969, pp.40–46.
6. Bignami, G. and H. Michalek. Cholinergic mechanisms and aversively motivated behaviors. In: *Psychopharmacology of Aversively Motivated Behavior,* edited by H. Anisman and G. Bignami. New York: Plenum Press, 1978, pp. 173–255.

7. Bignami, G., N. Rosic, H. Michalek, M. Milošević and G. L. Gatti. Behavioral toxicity of anticholinesterase agents: Methodological, neurochemical, and neuropsychological aspects. In: *Behavioral Toxicology*, edited by B. Weiss and V. G. Laties. New York: Plenum Press, 1975, pp. 155–215.
8. Browne, R. G. and D. S. Segal. Metabolic and experiential factors in the behavioral response to repeated amphetamine. *Pharmac. Biochem. Behav.* **6:** 545–552, 1977.
9. Bueno, O. F. A. and E. A. Carlini. Dissociation of learning in marihuana tolerant rats. *Psychopharmacologia* **25:** 49–56, 1972.
10. Campbell, J. C. and L. S. Seiden. Performance influence on the development of tolerance to amphetamine. *Pharmac. Biochem. Behav.* **1:** 703–708, 1973.
11. Cannizzaro, G., S. Nigito, P. M. Provenzano and T. Vitikova. Modification of depressant and disinhibitory action of flurazepam during short term treatment in the rat. *Psychopharmacologia* **26:** 173–184, 1972.
12. Cappell, H. and A. E. LeBlanc. Gustatory avoidance conditioning by drugs of abuse. In: *Food Aversion Learning*, edited by N. W. Milgram, L. Krames and T. M. Alloway. New York: Plenum Press, 1977, pp. 133–167.
13. Cappell, H., A. E. LeBlanc and S. Herling. Modification of the punishing effects of psychoactive drugs in rats by previous drug experience. *J. comp. physiol. Psychol.* **89:** 347–356, 1975.
14. Carlton, P. L. and D. L. Wolgin. Contingent tolerance to the anorexigenic effects of amphetamine. *Physiol. Behav.* **7:** 221–223, 1971.
15. Carro-Ciampi, G. and G. Bignami. Effects of scopolamine on shuttle-box avoidance and go-no go discrimination: Response-stimulus relationships, pretreatment baselines, and repeated exposure to drug. *Psychopharmacologia* **13:** 89–105, 1968.
16. Charney, N. H. and G. S. Reynolds. Development of behavioral compensation to the effects of scopolamine during fixed-interval reinforcement. *J. exp. Analysis Behav.* **8:** 183–186, 1965.
17. Charney, N. H. and G. S. Reynolds. Tolerance to the behavioral effects of scopolamine in rats. *Psychopharmacologia* **11:** 379–387, 1967.
18. Chippendale, T. J., G. A. Zawolkow, R. W. Russell and D. H. Overstreet. Tolerance to low acetylcholinesterase levels: Modification of behavior without acute behavioral change. *Psychopharmacologia* **26:** 127–139, 1972.
19. Colpaert, F. C., J. J. M. D. Kuyps, C. J. E. Niemegeers and P. A. J. Janssen. Discriminative stimulus properties of fentanyl and morphine: Tolerance and dependence. *Pharmac. Biochem. Behav.* **5:** 401–408, 1976.
20. Colpaert, F. C., C. J. E. Niemegeers and P. A. J. Janssen. Studies on the regulation of sensitivity to the narcotic cue. *Neuropharmacology* **17:** 705–713, 1978.
21. Colpaert, F. C., C. J. E. Niemegeers and P. A. J. Janssen. Nociceptive stimulation prevents development of tolerance to narcotic analgesia. *Eur. J. Pharmac.* **49:** 335–336, 1978.
22. Cook, L. and J. Sepinwall. Reinforcement schedules and extrapolation to humans from animals in behavioral pharmacology. *Fedn. Proc.* **34:** 1889–1897, 1975.
23. Corfield-Sumner, P. K. and I. P. Stolerman. Behavioral tolerance. In: *Contemporary Research in Behavioral Pharmacology*, edited by D. E. Blackman and D. J. Sanger. New York: Plenum Press, 1978, pp. 391–448.
24. Elsmore, T. F. The role of reinforcement loss in tolerance to chronic Δ^9-tetrahydrocannabinol effects on operant behavior of rhesus monkeys. *Pharmac. Biochem. Behav.* **5:** 123–128, 1976.
25. Ferraro, D. P. Effects of Δ^9-trans-tetrahydrocannabinol on simple and complex learned behavior in animals. In: *Current Research in Marijuana*, edited by M. F. Lewis. New York: Academic Press, 1972, pp. 49–95.
26. Fischman, M. W. and C. R. Schuster. Tolerance development to chronic methamphetamine intoxication in the rhesus monkey. *Pharmac. Biochem. Behav.* **2:** 503–508, 1974.
27. Fischman, M. W. and C. R. Schuster. Long-term behavioral changes in the rhesus monkey after multiple daily injections of *d*-methyl-amphetamine. *J. Pharmac. exp. Ther.* **201:** 593–605, 1977.
28. Florio, V., G. Bignami and V. G. Longo. EEG patterns during the behavioural desensitization to scopolamine in rats. *Int. J. Neuropharmac.* **8:** 405–411, 1969.
29. Frontali, M., L. Amorico, L. De Acetis and G. Bignami. A pharmacological analysis of processes underlying differential responding: A review and further experiments with scopolamine, amphetamine, lysergic acid diethylamide (LSD-25), chlordiazepoxide, physostigmine, and chlorpromazine. *Behav. Biol.* **18:** 1–74, 1976.
30. Hayes, R. L. and D. J. Mayer. Morphine tolerance: Is there evidence for a conditioning model? *Science* **200:** 343–344, 1978.
31. Janků, I. The influence of delayed and immediate exposure to trials upon the effect of chlorpromazine on conditioned avoidance behaviour. *Psychopharmacologia* **6:** 280–285, 1964.
32. Kalant, H., A. E. LeBlanc and R. J. Gibbins. Tolerance to, and dependence on, some non-opiate psychotropic drugs. *Pharmac. Rev.* **23:** 135–191, 1971.
33. Kokkinidis, L. and H. Anisman. Behavioural specific tolerance following chronic *d*- or *l*-amphetamine treatment: Lack of involvement of *p*-hydroxynorephedrine. *Neuropharmacology* **17:** 95–102, 1978.
34. Kokkinidis, L. and H. Anisman. Abatement of stimulus perseveration following repeated d-amphetamine treatment: Absence of behaviorally augmented tolerance. *Pharmac. Biochem. Behav.* **8:** 557–563, 1978.
35. Kokkinidis, L., J. Irwin and H. Anisman. Shock-induced locomotor excitation following acute and chronic amphetamine treatment. *Neuropharmacology* **18:** 13–22, 1979.
36. Larsen, F. F. and G. T. Pryor. Factors influencing tolerance to the effects of Δ^9-THC on a conditioned avoidance response. *Pharmac. Biochem. Behav.* **7:** 323–329, 1977.
37. LeBlanc, A. E., R. J. Gibbins and H. Kalant. Behavioral augmentation of tolerance to ethanol in the rat. *Psychopharmacologia* **30:** 117–122, 1973.
38. LeBlanc, A. E., R. J. Gibbins and H. Kalant. Generalization of behaviorally augmented tolerance to ethanol, and its relation to physical dependence. *Psychopharmacologia* **44:** 241–246, 1975.
39. Manning, F. J. Acute tolerance to the effects of delta-9-tetrahydrocannabinol on spaced responding by monkeys. *Pharmac. Biochem. Behav.* **1:** 665–671, 1973.
40. Manning, F. J. Role of experience in acquisition and loss of tolerance to the effect of Δ^9-THC on spaced responding. *Pharmac. Biochem. Behav.* **5:** 269–273, 1976.
41. Margules, D. L. and L. Stein. Increase of "antianxiety" activity and tolerance of behavioral depression during chronic administration of oxazepam. *Psychopharmacologia* **13:** 74–80, 1968.
42. McCleary, R. A. Response specificity in the behavioral effects of limbic system lesions in the cat. *J. comp. physiol. Psychol.* **54:** 605–613, 1961.
43. Meyers, B. Some effects of scopolamine on a passive avoidance response in rats. *Psychopharmacologia* **8:** 111–119, 1965.
44. Meyers, B. and M. A. Lazarus. Diminished responsivity on a passive avoidance task to a second administration of scopolamine. *Psychol. Rep.* **20:** 175–178, 1967.
45. Meyers, B. and R. C. Wilchin. Some effects of scopolamine on locomotor activity in rats. *Psychon. Sci.* **17:** 174–175, 1969.
46. Michalek, H., A. Meneguz, G. M. Bisso, G. Carro-Ciampi, G. L. Gatti and G. Bignami. Neurochemical changes associated with the behavioural toxicity of organophosphate compounds. In: *Advances in Pharmacology and Therapeutics, Vol. 9, Toxicology*, edited by Y. Cohen. Oxford: Pergamon Press, 1979, pp. 187–201.
47. Miksic, S. and H. Lal. Tolerance to morphine-produced discriminative stimuli and analgesia. *Psychopharmacology* **54:** 217–221, 1977.
48. Russell, R. W. Cholinergic substrates of behavior. In: *Cholinergic Mechanisms and Psychopharmacology*, edited by D. J. Jenden. New York: Plenum Press, 1977, pp. 709–731.

49. Russell, R. W., D. H. Overstreet, C. W. Cotman, V. G. Carson, L. Churchill, F. W. Dalglish and B. J. Vasquez. Experimental tests of hypotheses about neurochemical mechanisms underlying behavioral tolerance to the anticholinesterase, diisopropyl fluorophosphate. *J. Pharmac. exp. Ther.* **192:** 73–85, 1975.
50. Schuster, C. R., W. S. Dockens and J. H. Woods. Behavioral variables affecting the development of amphetamine tolerance. *Psychopharmacologia* **9:** 170–182, 1966.
51. Schuster, C. R. and J. Zimmerman. Timing behavior during prolonged treatment with *dl*-amphetamine. *J. exp. Analysis Behav.* **4:** 327–330, 1961.
52. Sepinwall, J., F. S. Grodsky and L. Cook. Conflict behavior in the squirrel monkey: Effects of chlordiazepoxide, diazepam, and N-desmethyldiazepam. *J. Pharmac. exp. Ther.* **204:** 88–102, 1978.
53. Siegel, S. Evidence from rats that morphine tolerance is a learned response. *J. comp. physiol. Psychol.* **89:** 498–506, 1975.
54. Siegel, S. Morphine analgesic tolerance: Its situation specificity supports a Pavlovian conditioning model. *Science* **193:** 323–325, 1976.
55. Siegel, S. Morphine tolerance acquisition as an associative process. *J. exp. Psychol.: Anim. Behav. Proc.* **3:** 1–13, 1977.
56. Siegel, S. Morphine tolerance: Is there evidence for a conditioning model? (Reply to Hayes and Mayer) *Science* **200:** 344–345, 1978.
57. Siegel, S., R. E. Hinson and M. D. Krank. The role of predrug signals in morphine analgesic tolerance: Support for a Pavlovian conditioning model of tolerance. *J. exp. Psychol.: Anim. Behav. Proc.* **4:** 188–196, 1978.
58. Thompson, D. M. Repeated acquisition of behavioral chains under chronic drug conditions. *J. Pharmac. exp. Ther.* **188:** 700–713, 1974.
59. Thornhill, J. A., M. Hirst and C. W. Gowdey. Variability in development of tolerance to repeated injections of low doses of *dl*-amphetamine in rats. *Can. J. Physiol. Pharmac.* **55:** 1170–1178, 1977.
60. Tormey, J. and L. Lasagna. Relation of thyroid function to acute and chronic effects of amphetamine in the rat. *J. Pharmac. exp. Ther.* **128:** 201–209, 1960.
61. Vogel, J. and B. A. Nathan. Reduction of learned taste aversions by pre-exposure to drugs. *Psychopharmacology* **49:** 167–172, 1976.
62. Weston, P. F. and D. H. Overstreet. Does tolerance develop to low doses of d- and l-amphetamine on locomotor activity in rats? *Pharmac. Biochem. Behav.* **5:** 645–649, 1976.

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Morphological Studies of Toxic Distal Axonopathy

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SCHAUMBURG, H. H. *Morphological studies of toxic distal anoxopathy.* NEUROBEHAV. TOXICOL. **1:** Suppl. 1, 187–188, 1979.—Distal axonopathy is the most common form of toxic injury to the peripheral nervous system. Morphological studies of the experimental distal axonapathies produced by acrylamide monomer and hexacarbons have lead to a reapparaisal of the dying-back hypothesis. These studies have also provided a rationale for many of the clinical findings in humans with distal axonopathies, and have been especially helpful in elucidating the effects of axonal neurotoxins on the central nervous system.

Toxic distal axonopathy Morphology

A RECENT classification of toxic disorders of the peripheral nervous system (PNS) has described three types of pathological reaction: nerve cell death (neuronopathy), segmental demyelination (myelinopathy) and distal axonal degeneration (distal axonopathy) [3]: Distal axonopathy is the principal focus of this report since it is the most common form of toxic disease of the nervous system.

In 1972, Dr. Spencer and I began our experimental animal studies of dying-back peripheral nerve degeneration produced by acrylamide. At that time, the dying-back hypothesis was a generally accepted concept in neuropathology. In brief, this idea suggested that a toxic derangement of neuronal metabolism would cause the nerve cell body to withdraw metabolic support from its axonal process so that degeneration would commence in the nerve terminal and, as neuronal metabolism became progressively impaired, axonal degeneration would move, in a seriate, retrograde (dying-back) fashion towards the cell body. If the intoxication was stopped it was assumed that axonal regeneration would commence and the animal (or human) would eventually recover. Neurons with the longest and largest diameter axons were thought to be most vulnerable to dying-back degeneration since they supported the greatest metabolic load.

Our studies have indicated that dying-back was an inaccurate and misleading term to describe the pathological process, and suggested that the designation central-peripheral distal axonopathy was more appropriate [5]. The need for this somewhat cumbersome term was emphasized by the fact that for many clinicians and neuropathologists, the appellation dying-back had become synonymous with peripheral neuropathy, despite the previous demonstration that varying degrees of CNS degeneration sometimes accompanied the peripheral changes. The need to give equal emphasis to the central and peripheral nervous system became self-evident when we demonstrated widespread distal axonal degeneration in the CNS occurring concurrently with similar changes in the PNS.

Experimental Studies of the PNS

Acrylamide. Our initial studies of acrylamide-induced PNS axonal degeneration were based on the commonly-held assumption that dying-back nerve fiber degeneration would begin in the nerve terminals and proceed in a seriate fashion up the fibers of the longest nerve (sciatic). Accordingly, we focused on the hindpaw of the cat where we examined the spatio-temporal pattern of axon degeneration in sensory terminals supplying pacinian corpuscles, the equatorial zone of nearby muscle spindles, and adjacent motor nerve terminals supplying extrafusal muscle fibers. Cats were daily injected with 1 mg/kg of an aqueous solution of acrylamide and tissue was obtained at stages of intoxication. Histopathological examination revealed that the most sensitive structure was the 7–11 μm axons supplying pacinian corpuscles which began to degenerate before the larger 11 μm axons supplying the annulospiral muscle spindle terminals. Both sensory endings were more vulnerable than the juxtaposed motor nerve terminals. Pacinian corpuscle terminals in the forepaw began to degenerate at the same time as those in the hindpaw. In addition, by exploring axonal regions proximal to the nerve terminals of pacinian corpuscles and muscle spindles, it was apparent that degeneration here sometimes preceded the onset of terminal degeneration. Thus, these studies with acrylamide directly challenged two of the basic tenets of the dying-back hypothesis, namely that axonal length and diameter dictated vulnerability and that degeneration began at the axon terminal before proceeding proximally [4].

Hexacarbons. The hexacarbon studies were undertaken after completion of the initial acrylamide investigation in order to evaluate further the dying-back process in the PNS.

Our studies with the hexacarbons (MBK, n-hexane and 2,5-hexanedione) were conducted in cats and rats, and employed various routes of administration, depending on the physical properties of the compound. Intoxicated cats and rats lost weight, then developed an unsteady gait and hindlimb foot drop. With continued intoxication forelimb distal weakness appeared and, after very prolonged intoxication, animals became quadriparetic and were unable to sit or stand. At no stage did they display truncal ataxia or head tremor, clinical features which had been prominent in cats intoxicated with acrylamide. Histopathological studies revealed that the large myelinated fibers of the tibial nerve branches to the calf muscles displayed paranodal giant axonal degeneration before similar changes were evident in the more distal plantar nerve fibers in the hindpaw. The nerves to the calf muscles contain the largest diameter myelinated axons in the hindlimb, and their special sensitivity to hexacarbons suggested that axon diameter might play a major role in determining vulnerability. The importance of axonal length was also apparent because the hindlimbs, containing long nerves, were consistently involved before forelimbs, supplied with shorter nerves. Surprisingly, regenerating fibers were also seen scattered among the degenerating axons during the course of intoxication. Another important observation in the hexacarbon studies was that degeneration did not begin in the terminal and travel steadily up the nerve fiber, but rather began in a multifocal pattern on the proximal sides of multiple nodes of Ranvier in distal nerve fibers. These findings confirmed our earlier observations in acrylamide animals, namely, that the axonal degeneration in dying-back neuropathies did not begin at the terminal and progress toqards the cell body in a seriate fashion, as the term dying-back had implied [6].

Experimental Studies of the CNS

In contrast to the PNS changes, the distribution of CNS axonal degeneration produced by acrylamide and the hexacarbons compounds appeared identical. A striking and consistent finding in the hexacarbon neuropathies was the development of axonal degeneration in certain areas of the central nervous system at the same time changes were first noted in the most vulnerable areas of the PNS. In general, these changes were found in the distal regions of long spinal nerve tracts and later, in shorter CNS pathways. As in the PNS, tractal degeneration appeared to spread toward the neuron cell body with continued intoxication. The distribution of the changes in asymptomatic, hexacarbon-intoxicated animals showed that the rostral regions of long, ascending sensory tracts (gracile fasiculus, dorsal spinocerebellar) and caudal regions of long, descending motor pathways (corticospinal) were first affected. The sensory pathways were more sensitive than the descending fibers, and the gracile nucleus and mossy fibers and white matter of the anterior cerebellar vermis were the first CNS structures to display axonal swellings. In animals with more prolonged intoxication and moderate clinical impairment these same areas displayed an advanced stage of axonal degeneration accompanied by breakdown of the myelin sheath and a mild astrocytic proliferation [7]. In cats with very prolonged intoxication, and severe weakness in all extremities, axonal swellings were present in the distal optic tract, superior colliculus, lateral geniculate body and mammillary bodies [2]. These studies confirmed many ideas about the dying-back process that had been drawn from earlier studies of the spatio-temporal pattern in the PNS. The distal regions of long and large CNS fibers were susceptible to degeneration, and axon length appeared an important factor in determining vulnerability.

Recovery from Hexacarbon Intoxication

Several of the cats with very prolonged intoxication were allowed to recover for periods of up to two years. These animals gradually regained weight, became mobile and, after a year's recovery, appeared to have regained strength in all extremities. However, on attempting to walk, all animals manifested stiff-legged, unsteady hindlimb movements that resulted in a swaying motion of the pelvis. Attempts at running resulted in a reeling movement and the animals fell frequently to either side. Histopathological examination revealed that the gracile nuclei consistently displayed the most severe changes in these recovered animals with a striking axonal loss, diffuse gliosis and loss of neurons. In the lateral columns of the lumbar spinal cord there was moderate axonal loss accompanied by mild gliosis, and gliosis and fiber loss were prominent in the white matter of the anterior vermis of the cerebellum. Based on these data we concluded that the degeneration in the corticospinal tracts and cerebeller vermis probably accounted for the residual spastic-ataxic gait in these animals.

Human Correlation

In brief, the clinical findings in humans with acrylamide and n-hexane intoxication correlate well with the data obtained from animal studies. Humans with acrylamide intoxication initially develop a severe loss of peripheral vibratory sensation, numbness and an unsteady, ataxic gait: while those with chronic n-hexane exposure display distal symmetrical weakness and sensory loss. Individuals recovering from severe n-hexane peripheral neuropathy have developed residual spasticity, and visual impairment has been demonstrated following prolonged exposure to n-hexacarbons [1,8].

REFERENCES

1. Koroblin, R., A. K. Asbury, A. J. Sumner and S. L. Niesen. Glue-sniffing neuropathy. *Archives of Neurol.* **32:** 158–162, 1975.
2. Schaumburg, H. H. and P. S. Spencer. Environmental hydrocarbons produce degeneration in cat hypothalamus and optic tract. *Science* **199:** 199–200, 1978.
3. Schaumburg, H. H. and P. S. Spencer. Toxic neuropathies. *Neurology* **29:** 429–431, 1979.
4. Schaumburg, H. H., H. M. Wisniewski and P. S. Spencer. Ultrastructural studies of the dying-back process. I. Peripheral nerve terminal and axonal degeneration in systemic acrylamide intoxication. *Journal of Neuropath. Exp. Neurology* **33:** 260–295, 1974.
5. Spencer, P. S. and H. H. Schaumburg. Central-peripheral distal axonopathy—the pathology of dying-back polyneuropathies. In: *Progress in Neuropathology*, Vol. 3, edited by H. Zimmerman. New York: Grune and Stratton, 1977, pp. 253–295.
6. Spencer, P. S. and H. H. Schaumburg. Ultrastructural studies of the dying-back process. III. The evolution of experimental peripheral giant axonal degeneration. *Journal of Neuropath. Exp. Neurol.* **36:** 276–299, 1977.
7. Spencer, P. S. and H. H. Schaumburg. Ultrastructural studies of the dying-back process. IV. Differential vulnerability of PNS and CNS fibers in experimental central-peripheral distal axonopathies. *Journal of Neuropath. Exp. Neurol.* **36:** 300–320, 1977.
8. Yamamura, Y. n-Hexane polyneuropathy. *Folia psychiat. Neurol. Jap.* **23:** 45–57, 1969.

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Cellular Responses to Neurotoxic Compounds of Environmental Significance

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SPENCER, P. S. *Cellular responses to neurotoxic compounds of environmental significance.* NEUROBEHAV. TOXICOL. **1:** Suppl. 1, 189–191, 1979.—Many neurotoxic chemicals of environmental significance can be conveniently classified according to their cellular site of action in the nervous system. This paper considers neurotoxins which damage the nerve cell body (neuronopathy), its axonal process (axonopathy), or the myelin sheath which segmentally enwraps the myelinated axon (myelinopathy). Each of these three conditions can be reproduced in experimental animals for study of the mechanisms and consequences of neurotoxic damage. Detailed morphological examination of toxic distal axonopathies have stimulated biochemical studies which promise to yield a precise explanation of the molecular basis for this common type of neurotoxic disease. It seems possible that a precise description of the molecular basis for toxic distal axonal degeneration is within sight.

Neurotoxins Neuronopathy Axonopathy Myelinopathy

MANY neurotoxic chemicals of environmental significance can be conveniently classified according to their cellular site of action in the nervous system [14]. This paper considers neurotoxins which damage the nerve cell body (neuronopathy), its axonal process (axonopathy), or the myelin sheath which segmentally enwraps the myelinated axon (myelinopathy). Each of these three conditions can be reproduced in experimental animals for study of the mechanisms and consequences of neurotoxic damage. For example, the rat develops a neuronopathy restricted to neurons in peripheral ganglia following administration of Adriamycin [2], a central and peripheral myelinopathy from acetyl ethyl tetramethyl tetralin [12], and a central-peripheral distal axonopathy from hexacarbon solvents, acrylamide or carbon disulfide [9].

TOXIC NEURONOPATHY

The anthracycline antibiotic, Adriamycin, occupies an increasingly important position in the treatment of malignant tumors. Administered intravenously to cancer patients, the drug binds to nucleic acid species and inhibits the growth of rapidly dividing malignant cells. Although neurological disease has not been reported in patients treated with Adriamycin, this is a reproducible response in the rat. After a single, intravenous injection of 10 mg/kg, animals develop an abnormal limb posture and later die. The morphological substrate of this neurological illness has been determined by light and electron microscope examination of the peripheral nervous system. Fluorescence studies [6] have demonstrated that Adriamycin binds to the cell nuclei of sensory and autonomic ganglia, sites where the blood-nerve barrier is normally porous [4]. Within a few hours, histological changes appear in neuronal nuclei of sensory ganglia as a clumping and focal clearing of chromatin. After several days, many of the more severely affected, larger neurons undergo nuclear and cytoplasmic degeneration, presumably because the anabolic machinery of the cell has been shut down. The surrounding satellite cells, which also bind Adriamycin, but do not undergo breakdown, cluster to occupy the degenerative site of the neuron. This selective loss of the nerve cell body is accompanied by a rapid, secondary breakdown of the axon—both the centrally-directed process to the spinal cord and the peripheral axon supplying sensory terminals such as stretch receptors in the muscle spindle. Deficient spindle sensory input probably accounts in significant part for the bizarre limb posturing seen in sensory neuronopathies. The limbs are not weak since Adriamycin fails to damage the motor nerve cell or axon, presumably because the former is located in the spinal cord and protected from the drug by the blood-brain barrier.

In summary, Adriamycin produces a primary degeneration of neurons in sensory and autonomic ganglia, secondary breakdown of the axon, and permanent denervation of certain sensory receptors. Although other examples of toxic sensory neuronopathy can be cited, e.g., methyl mercury [4], such diseases are surprisingly rare in view of the ease with which circulating toxins can gain direct access to the neurons of peripheral ganglia.

TOXIC MYELINOPATHY

Primary breakdown of myelin is a more common response to systemic intoxication. Alkyl tins, cuprizone, hexachlorophene, tellurium and acetyl ethyl tetramethyl tetralin (AETT) are examples of myelinotoxins [1]. The fragance comound AETT produces a progressive neurological disease in chronically intoxicated animals. When AETT is daily administered to the rat, either by an oral or percutaneous route, the animal gradually develops a bizarre, arched-back posture and subsequently develops limb weakness. Both of these signs are preceeded by hyperirritability and an extraordinary blue discoloration of internal organs including the brain.

The morphological substrate of AETT intoxication is complex, comprising an early, slow progressive and widespread ceroid neuronopathy, and a later, primary demyelination. Demyelination begins in the large diameter fibers of central and peripheral nervous tissue. In the latter, affected myelin segments undergo ballooning to form large intramyelinic vacuoles in which phagocytic cells eventually reside. These cells selectively removed the damaged myelin sheath, leaving lengths of axons denuded but intact. Schwann cells then envelop the bare portions of axon and produce short, remyelinated segments. This combination of demyelination and remyelination, structural events associated with blockade and restoration of nerve impulse transmission can account for the progressive limb weakness which eventually reaches a plateau.

The cellular mechanisms of AETT myelinopathy have yet to be resolved. However, several morphological observations point to the myelin sheath rather than the myelin-producing cell (Schwann cell or oligodendrocyte) as the likely locus of toxic damage.

TOXIC DISTAL AXONOPATHY

Damage to the axon of the nerve cell, especially the distal ends of long and large axons (distal axnonopathy), probably constitutes the single most common neuropathological response to neurotoxic agents. Prolonged, low-level intoxication is usually required to produce clinical disease (peripheral neuropathy) which is typically expressed in the form of distal, symmetrical hindlimb weakness and sensory loss. (Schaumburg, *this volume*.)

The distal axonopathy produced by neurotoxic hexacarbons [11] has been studied most thoroughly. The first identifiable pathological event is a focal axonal swelling which appears on the proximal side of one or more nodes of Ranvier in the sub-terminal parts of the nerve fibers. These axonal swellings contain large numbers of neurofilaments and other organelles which normally move along the axon. Similar changes may be found in animals intoxicated with acrylamide or carbon disulfide. Axoplasmic organelles seem to accumulate at nodes of Ranvier because the axonal transport systems are locally defective. The portion of axon below the position of transport blockade then undergoes degeneration, thereby its sensory or motor innervation.

The neurotoxic property of the hexacarbon solvents is attributable, in large part, to the water-soluble neurotoxic metabolite 2,5-hexanedione [3]. This compound is more potent than its related metabolites 2,5-hexanediol, 5-hydroxy-2-hexanone, 2-hexanol, 2-hexanone (M*n*BK), or *n*-hexane, and consistently appears as a metabolite of all the other compounds both *in vivo* and in tissue culture models of hexacarbon neuropathy [7,15]. The neurotoxic property of 2,5-hexanedione (2,5-HD) is associated with the gamma spacing of the carbonyl groups; other compounds of longer chain length and non-symmetrical gamma carbonyl groupings are also neurotoxic. By contrast, compounds which lack the gamma spacing of the carbonyl groups (e.g., 2,4-hexanedione) fail to exhibit neurotoxicity [7,9].

Knowledge of the mechanism of cellular damage in distal axonopathies has advanced considerably in recent years. The concept that toxic chemicals damage the nerve cell body, causing long and large axons to die-back from their distal ends, has been replaced by a theory of direct damage to the nerve fiber. Organophosphates, isoniazid, acrylamide, disulfiram and hexacarbons are all believed to damage the nerve fiber directly. Direct evident for this thesis exists in the case of diisofluorophosphate [5] and 2,5-hexanedione [8]. When 2,5-HD is applied repetitively to a peripheral nerve, giant axonal swellings of the type seen in systemic intoxication develop beneath the site of application. No such dwellings appear when non-neurotoxic 2,4-hexanedione is used.

The biochemical lesion(s) underlying distal axonopathy remain to be elucidated. Especially puzzling is how a number of chemically distinct compounds can induce closely similar patterns of cellular breakdown. For example, the hexacarbons, acrylamide and carbon disulfide (CS_2) are all capable of producing giant axonal swelling as a prelude to degeneration. One explanation may be that such compounds act at nearby or related sites in metabolic pathways, the net biochemical defect being common for all three compounds [13]. Intense interest is currently focussed on glycolysis and associated energy tranformation pathways because these are implicated in toxic and vitamin deficiency states associated with distal axonopathy. 2,5-HD, CS_2 and acrylamide each inhibit *in vitro* the major glycolytic enzymes glyceraldehyde phosphate dehydrogenase and phosphofructokinase, the degree of inhibition being dependent both on the concentration of toxin and the duration of preincubation of toxin and enzyme [9]. A comparable inhibition of enzymes in the nerve fiber axon presumably would raise the demand for their replacement by the nerve cell body. Failure to resupply the entire axon would leave distal regions with an inadequate of glycolytic enzymes resulting in a depletion of energy supplies in this region. This may produce a blockade of energy-dependent axonal transport at energy-intensive nodes of Ranvier, and initiate the sequence of pathological changes which leads to distal nerve fiber degeneration [13].

CONCLUSIONS

The application of advanced investigative techniques has provided neuropathological assays to detect very early structural changes in the nervous system [10], allowing one to identify and distinguish between three major types of neurotoxic disease: neuronopathy, myelinopathy and axonopathy. Detailed morphological examination of toxic distal axonopathies have stimulated biochemical studies which promise to yield a precise explanation of the molecular basis for this common type of neurotoxic disease. It seems possible that a precise description of the molecular basis for toxic distal axonal degeneration is within sight.

REFERENCES

1. Cammer, W. Toxic demyelination: biochemical studies and hypothetical mechanisms. In: *Experimental and Clinical Neurotoxicology*, edited by P. S. Spencer and H. H. Schaumburg. Baltimore, MD: Williams and Wilkins, 1980.
2. Cho, E-S., P. S. Spencer and B. S. Jortner. Doxorubicin. In: *Experimental and Clinical Neurotoxicology*, edited by P. S. Spencer and H. H. Schaumburg. Baltimore, MD: Williams and Wilkins, 1980.

3. DiVincenzo, G. D., M. L. Hamilton, C. J. Kaplan and J. Dedinas. Characterization of the metabolites of methyl *n*-butyl ketone. In: *Experimental and Clinical Neurotoxicology*, edited by P. S. Spencer and H. H. Schaumburg. Baltimore, MD: Williams and Wilkins, 1980.
4. Jacobs, J. M. Vascular permeability and neural injury. In: *Experimental and Clinical Neurotoxicology*, edited by P. S. Spencer and H. H. Schaumburg. Baltimore, MD: Williams and Wilkins, 1980.
5. Lowndes, H. E. and T. Baker. Toxic site of action in distal axonopathies. In: *Experimental and Clinical Neurotoxicology*, edited by P. S. Spencer and H. H. Schaumburg. Baltimore, MD: Williams and Wilkins, 1980.
6. Mendell, J. R. and Z. Sahenk. Interference of neuronal processing and axoplasmic transport by toxic chemicals. In: *Experimental and Clinical Neurotoxicology*, edited by P. S. Spencer and H. H. Schaumburg. Baltimore, MD: Williams and Wilkins, 1980.
7. O'Donoghue, J. L. and W. J. Krasavage. Identification and characterization of M*n*BK neurotoxicity in laboratory animals. In: *Experimental and Clinical Neurotoxicology*, edited by P. S. Spencer and H. H. Schaumburg. Baltimore, MD: Williams and Wilkins, 1980.
8. Politis, M. J., R. G. Pellegrino and P. S. Spencer. Ultrastructural studies of the dying-back process. V. Axonal neurofilaments accumulate at sites of 2,5-hexanedione application: evidence for nerve fiber dysfunction in experimental hexacarbon neuropathy. *J. Neurocytol.*, submitted for publication.
9. Sabri, M. I. and P. S. Spencer. Toxic distal axonopathy: biochemical studies and hypothetical mechanisms. In: *Experimental and Clinical Neurotoxicology*, edited by P. S. Spencer and H. H. Schaumburg. Baltimore, MD: Williams and Wilkins, 1980.
10. Spencer, P. S., M. C. Bischoff and H. H. Schaumburg. Neuropathological methods for the detection of Neurotoxic Disease. In: *Experimental and Clinical Neurotoxicology*, edited by P. S. Spencer and H. H. Schaumburg. Baltimore, MD: Williams and Wilkins, 1980.
11. Spencer, P. S., D. Couri and H. H. Schaumburg. *n*-Hexane and methyl *n*-butyl ketone. In: *Experimental and Clinical Neurotoxicology*, edited by P. S. Spencer and H. H. Schaumburg. Baltimore, MD: Williams and Wilkins, 1980.
12. Spencer, P. S., G. Foster, A. B. Sterman and D. Horoupian. Acetyl ethyl tetramethyl tetralin. In: *Experimental and Clinical Neurotoxicology*, edited by P. S. Spencer and H. H. Schaumburg. Baltimore, MD: Williams and Wilkins, 1980.
13. Spencer, P. S., M. I. Sabri, H. H. Schaumburg and C. Moore. Does a defect of energy metabolism in the nerve fiber underlie axon degeneration in polyneuropathies? *Annls Neurol.* **5**: 501, 1979.
14. Spencer, P. S. and H. H. Schaumburg. Classification of neurotoxic disease: A morphological approach. In: *Experimental and Clinical Neurotoxicology*, edited by P. S. Spencer and H. H. Schaumburg. Baltimore, MD: Williams and Wilkins, 1980.
15. Veronesi, B., E. R. Peterson and P. S. Spencer. Reproduction and analysis of M*n*BK neuropathy in organotypic tissue culture. In: *Experimental and Clinical Neurotoxicology*, edited by P. S. Spencer and H. H. Schaumburg. Baltimore, MD: Williams and Wilkins, 1980.

Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function
Neurobehavioral Toxicology, Vol. 1, Suppl. 1, pp. 193–206. ANKHO International Inc., 1979

Physiological and Neurobehavioral Alterations During Development in Lead Exposed Rats

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FOX, D. A. *Physiological and neurobehavioral alterations during development in lead exposed rats.* NEUROBEHAV. TOXICOL. **1:** Suppl. 1, 193–206, 1970.—Neonatal rats were exposed to lead (Pb) from parturition to weaning via the milk of dams which consumed 0 (tap water), 0.02% or 0.2% PbAc$_2$ solutions. To determine if this regimen altered physiological and neurobehavioral development, responses to a battery of sensory-motor tests were evaluated during maturation and as adults. The tests were: visual evoked responses (VER), temperature regulation, maximal electroshock seizure patterns, reflex patterns, and neuromuscular performance. Overall results revealed that the Pb-exposed group compared to controls exhibited delayed maturation, altered developmental patterns and long-term CNS disturbances. Additionally, low-level strychnine administration during development caused additive interactions with both Pb groups, uncovering subtle effects of toxicant exposure. These sensitive and quantifiable techniques proved useful for assessing CNS functioning following perinatal insult, and except for the VER, are simple to conduct and cost efficient because they require a minimal amount of personnel training, equipment cost and time invested per animal. These screening tests also suggest further areas of study and may indicate the mechanism(s) responsible for the deficit.

Lead toxicity   Visual evoked response development   Seizure development   Behavioral development   Temperature regulation development   Behavioral toxicology   Drug-toxicant interactions   Screening tests

DESCRIPTIONS of the neurologic sequelae of lead (Pb) encephalopathy in children frequently include reports of visual-motor deficits, recurrent grand mal seizures, retarded behavioral development and electroencephalographic abnormalities [12, 57, 73, 80]. However, few investigators have examined the extent of functional impairment in the presumed asymptomatic pediatric population despite reports of neurobehavioral deficits in this population [6,88] and in animals following low-level Pb exposure during development [9, 13, 26, 27, 32, 54, 55, 74].

Sensory, motor and integrative systems function differently in the neonate and in the adult. During the early postnatal or preweaning period in the rat, a time when the most dynamic changes are occurring in the biochemical and morphological components of the nervous system, the CNS appears to be particularly sensitive to toxic insult [17,38]. During this developmental period different neural substrates, responsible for the physiological and behavioral activity of the organism, possess different spatio-temporal rates of maturation [37,77]. The rates at which these dynamic changes occur are subject, within certain genetic limits, to modification by both internal and external environmental influences. Recent evidence [16, 26, 27, 55, 64, 75] demonstrates that subtle functional impairment occurs in animals subjected to toxic insult during the prenatal and or early postnatal period at exposure levels below those producing observable abnormalities.

This report focuses on the physiological and neurobehavioral alterations during development resulting from low-level neonatal Pb exposure. The primary purpose of these studies was to determine the effects of this exposure protocol on the ontogeny of (1) visual evoked responses (VER), (2) temperature regulation, (3) maximal electroshock seizure (MES) patterns, (4) reflex patterns and neuromuscular performance, and (5) somatic indices of development.

This battery of tests examining the sensory, motor and integrative systems of the neonate was chosen to examine the specificity of effect and to determine at what age the Pb treatment effects first appeared. In order to study the interactive effects of Pb exposure and additional stressors, to examine possible mechanisms of action of Pb, and to validate our assessment techniques, some pups were injected with a subconvulsive dose of strychnine prior to testing. Strychnine, a neuropharmacological agent with known mechanisms of action [21,28], has been used to assess the maturation of inhibitory synapses [61,92].

Pb AND VER

The neurophysiological development of the visual system following neonatal Pb-exposure was examined because visual-motor and visual-perceptual deficits have been frequently reported in children following Pb poisoning [8, 12, 57] and because long-term deficits in visual discrimination learning have been reported in animals after early Pb-exposure [9,13]. The first appearance of the VER establishes the time at which this sensory system begins to function. During CNS development two components of the VER emerge in an orderly sequence. According to one group of investigators [65] the ontogenetically early appearing, long-latency, positive-negative complex (P2N2) is a manifestation of activity in the nonspecific corticipetal radiation (superior colliculus and pretectal region of the midbrain), whereas the

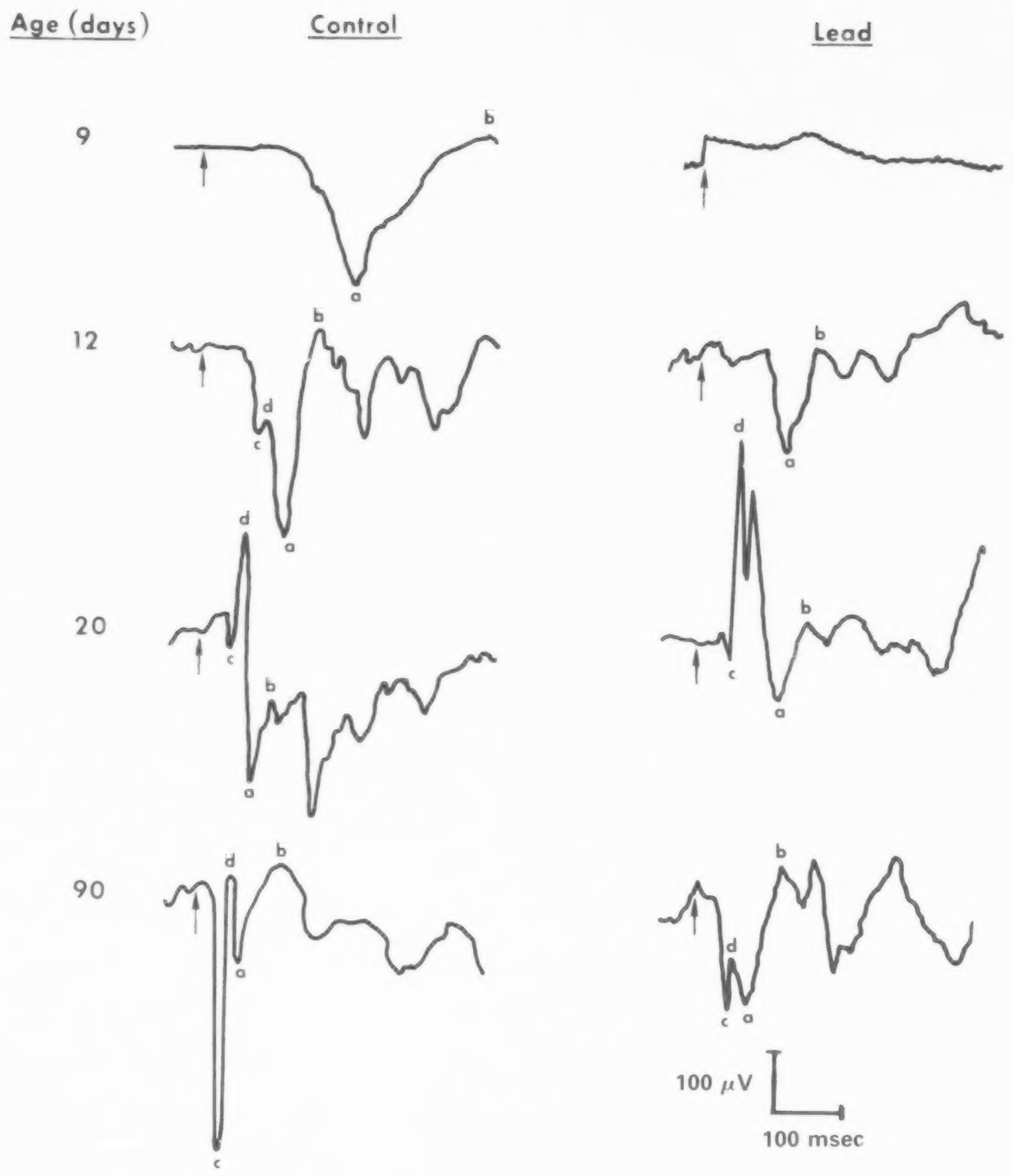

FIG. 1. Patterns of visual evoked response in developing and adult rats following neonatal Pb exposure. Dams' were consuming 0.2% PbAc or tap water. Average form of 20 VERs. Arrows indicate stimulus onset. Positive polarity downwards. a = P2; b = N2; c = P1; d = N1.

ontogenetically later-appearing, positive-negative complex (P1N1) reflects activity in the specific sensory system (lateral geniculate nucleus). Thus this method may provide a means for differentiating lesion sites in the visual system following perinatal insult [26, 49, 66, 89]. By measuring the change in peak latency of each component of the VER with age, the rate at which the neural generating systems responsible for that component matures can be compared between groups following exposure to a toxic agent [59, 65, 67].

In the first study, conducted in collaboration with Drs. J. P. Lewkowski and G. P. Cooper, the effects of exposing Long-Evans neonatal rats to Pb via dams' milk (dams'

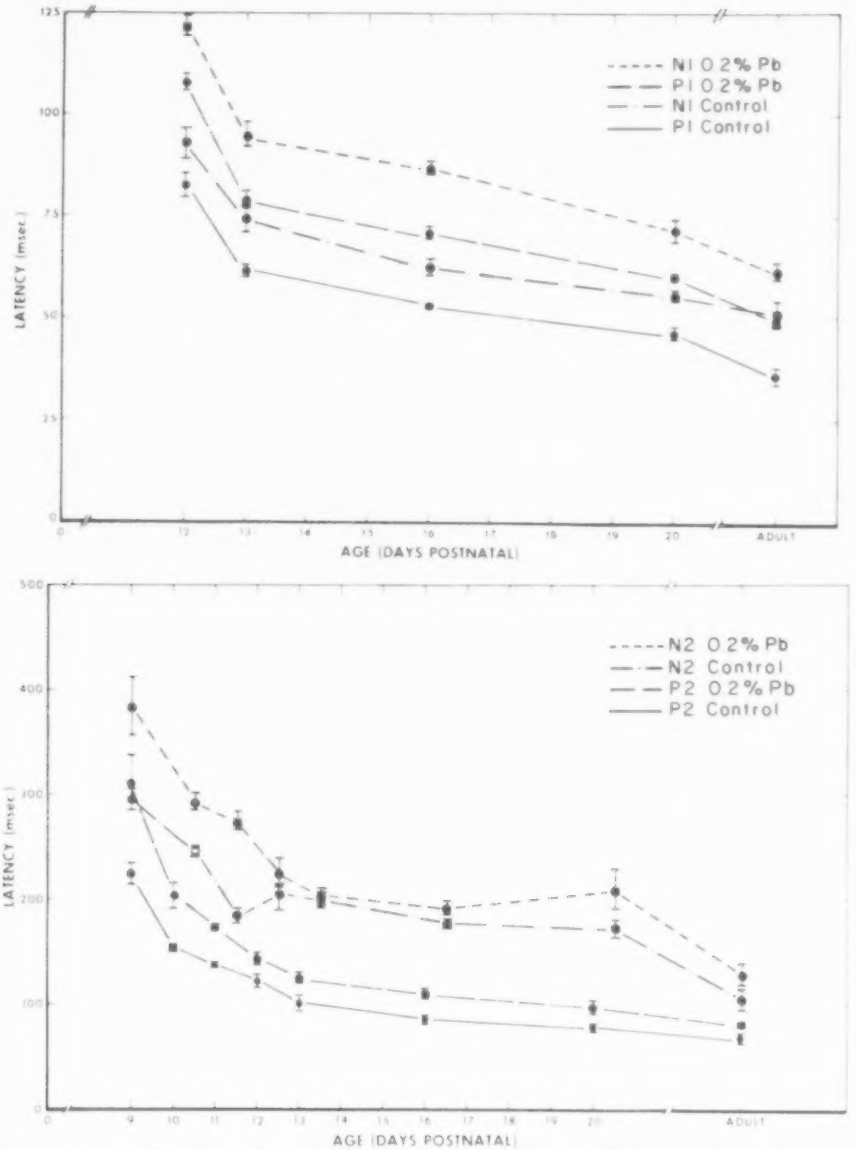

FIG. 2. Mean peak latencies of visual evoked response in developing and adult rats following neonatal Pb exposure. Points presented indicate the means ± SE for six animals per treatment per age. The data were analyzed by an ANOVA and the Student t-test and are presented in the text of paper. (Top) Mean ± SE for P1 and N1. (Bottom) Mean ± SE for P2 and N2.

consuming 0.2% PbAc$_2$) from parturition to weaning on brain electrical activity were determined in acute experiments performed on 84 male rats on Days 9–13, 16 and 20 and on 12 adult male rats (90–100 days old) [26]. Rats were anesthetized by pentobarbital sodium (30 mg/kg, IP) and the instantaneous power spectrum was monitored on-line (Nicolet Spectrum Analyzer, Model UA-500A), in order to maintain the animals in a state of moderate to light anesthesia. VERs were recorded on the visual cortex following a brief (10 μsec) light flash produced by a Grass photostimulator. To evaluate the responses, 20 VERs were averaged (Mnemotron CAT, Model 400B) to produce one averaged VER. Five such summations were made for each animal. An analysis of variance (ANOVA) with repeated measures showed no significant time effect with respect to the five summations, so the average of the five summations was used. The latencies of each component (P1, N1, P2, N2) were measured at the point of greatest amplitude and calculated from the time of

TABLE 1

LATENCIES (MSEC) OF VER COMPONENTS IN DEVELOPING AND ADULT RATS FOLLOWING NEONATAL LEAD EXPOSURE[a]

Age (Days)	Treatment	P_1	N_1	P_2	N_2
9	C	—	—	224.6 ± 9.6	296.2 ± 9.7
	0.2% Pb	—	—	311.7 ± 26.3^{bd}	384.9 ± 26.2^{bd}
10	C	—	—	153.7 ± 3.2	246.8 ± 5.1
	0.2% Pb	—	—	203.9 ± 12.2^{e}	294.2 ± 8.1^{e}
11	C	—	—	138.1 ± 1.8	184.8 ± 7.1
	0.2% Pb	—	—	174.2 ± 3.3^{f}	275.9 ± 7.6^{f}
12	C	82.4 ± 3.0	92.8 ± 3.8	122.7 ± 6.4	206.6 ± 17.1
	0.2% Pb	107.8 ± 2.1^{cf}	121.7 ± 2.5^{cf}	134.6 ± 6.2	277.4 ± 13.5
13	C	61.5 ± 1.7	74.2 ± 3.2	102.5 ± 8.0	201.1 ± 5.8
	0.2% Pb	78.9 ± 2.1^{f}	95.1 ± 2.8^{f}	126.2 ± 4.6^{d}	203.4 ± 8.2
16	C	53.1 ± 0.5	64.7 ± 1.8	86.3 ± 3.7	177.6 ± 4.3
	0.2% Pb	61.0 ± 1.6^{f}	86.8 ± 1.6^{f}	110.5 ± 3.8^{e}	193.2 ± 5.8^{d}
20	C	46.2 ± 1.3	55.8 ± 1.3	78.7 ± 3.8	172.7 ± 8.2
	0.2% Pb	60.4 ± 0.5^{f}	71.8 ± 2.5^{f}	99.6 ± 6.4^{e}	211.3 ± 19.1
90–100	C	36.0 ± 2.0	51.6 ± 2.9	68.7 ± 4.5	106.5 ± 10.6
	0.2% Pb	49.6 ± 1.8^{f}	61.8 ± 1.7^{d}	81.9 ± 0.9^{d}	129.7 ± 11.0

[a] Values presented are means ± SE for 6 animals per treatment per age. The data were analyzed by four separate two-way ANOVAs. Significant effects from these ANOVAs are presented in the text. Comparisons among treatment cell means are presented here using the Student *t*-test. Significant differences between means are indicated by superscripts d, e and f.

[b] Three of six animals exhibited no response.

[c] Four of six animals exhibited no response.

[d] Significantly different from control value at $p<0.05$.

[e] Significantly different from control value at $p<0.01$.

[f] Significantly different from control value at $p<0.001$.

stimulus onset. For this experiment and all others reported herein, the experimenter was unaware of the animals' experimental condition.

Rats exposed to Pb compared to controls showed no changes in growth rate, brain wet weight, or brain dry weight at any of the ages examined or in age of eye opening. At 21 days of age, the Pb concentration in the blood and brain of controls were 6 ± 1 and 7 ± 1 μg%, respectively, while Pb-exposed rats had 65 ± 9 and 53 ± 4 μg%, respectively. By Day 90, the Pb concentrations in blood and brain of both groups were approximately 6 and 12 μg%, respectively [26].

The effects of Pb on the VER were: (1) to delay the appearance of each component, (2) to alter the waveform, and (3) to increase the latencies of each component (Figs. 1 and 2). For example, on Day 9 when 100% of the control pups developed the initial long-latency P2N2 complex, only 50% of the Pb-exposed pups exhibited this response. Similarly on Day 12, when 100% of the controls developed the short-latency P1N1 complex, only 33% of the Pb-exposed pups exhibited this response. Figure 1 (Lead, Days 20 and 90) is a typical example which illustrates the altered waveform present in approximately 30% of the Pb-exposed animals between Days 16 and 90. A similar alteration, attributed to a demyelination of the rapidly conducting rate nerve fibers, has been reported to occur in adult rats dosed subacutely with an organic mercurial compound [41]. The latencies of the VER components were all significantly longer (about 30%) in the Pb group compared to controls as analyzed by an overall ANOVA.

Post-hoc comparisons of the means for the P1 and N1 components in the Pb group compared to controls revealed highly significant differences at all ages, while similar comparisons for the P2 and N2 components revealed differences at all ages except Day 12 for the P2 wave and Days 12, 13, 20 and 90 for the N2 wave (Table 1). The latter was due to the wide variability found in this component.

The data from this study demonstrates that low-level developmental Pb exposure in age- and weight-matched neonatal rats results in immediate and long-term neurophysiological disturbances in a primary sensory system. The effect upon the specific projection system probably represents a decreased conduction velocity within the CNS. Similar findings in peripheral nerves have been reported in industrial lead workers with blood Pb concentrations below 70 μg/100 ml [69]. An effect upon the nonspecific projection system may have more serious implications for the long term health of the animal, since it is well established that this nonspecific system plays a fundamental role in the elaboration of arousal, learning, attention, perception, emotional behavior, as well as in the control of neocortical electrical rhythms [42].

The long-term (90–100 days of age) effects observed in

TABLE 2

PREWEANING BODY WEIGHTS (G) OF LEAD-EXPOSED AND SEIZURE-TESTED RATS

	3	7	10	14	18	21
No Pb						
No MES, No Strychnine	9.0 ± 0.2	14.3 ± 0.5	19.7 ± 0.6	27.6 ± 0.7	36.7 ± 0.9$^{d'f'}$	43.8 ± 1.0$^{d'}$
MES	8.7 ± 0.2	14.6 ± 0.4	20.0 ± 0.5	27.3 ± 0.6	34.6 ± 0.7	41.6 ± 0.8
MES + Strychnine	8.5 ± 0.2	14.2 ± 0.5	19.9 ± 0.6	26.9 ± 0.7	34.7 ± 0.8	40.6 ± 1.0
All Pups	8.7 ± 0.1	14.4 ± 0.3	19.8 ± 0.3	27.2 ± 0.4^{b}	35.0 ± 0.5^{b}	42.2 ± 0.6^{b}
0.02% Pb						
No MES, No Strychnine	9.6 ± 0.2	15.1 ± 0.4	19.6 ± 0.5	27.2 ± 0.7	35.0 ± 0.8	43.6 ± 1.0$^{e'}$
MES	9.7 ± 0.2	14.6 ± 0.4	20.1 ± 0.4	27.2 ± 0.6	34.5 ± 0.7	41.5 ± 0.8
MES + Strychnine	9.6 ± 0.2	14.7 ± 0.3	20.0 ± 0.4	27.1 ± 0.5	34.3 ± 0.7	42.3 ± 0.8$^{f'}$
All Pups	9.6 ± 0.1	14.8 ± 0.2	19.9 ± 0.1	27.2 ± 0.3^{c}	34.6 ± 0.5^{c}	42.6 ± 0.5^{c}
0.2% Pb						
No MES, No Strychnine	9.2 ± 0.2	14.4 ± 0.3	19.5 ± 0.5	25.8 ± 0.5	33.2 ± 0.7	41.8 ± 1.0
MES	9.3 ± 0.2	14.5 ± 0.4	18.9 ± 0.4	25.4 ± 0.4	32.7 ± 0.5^{f}	38.6 ± 0.7^{def}
MES + Strychnine	9.1 ± 0.2	14.2 ± 0.4	18.6 ± 0.5	25.2 ± 0.5	31.8 ± 0.6^{d}	38.6 ± 0.9^{def}
All Pups	9.2 ± 0.1	14.4 ± 0.2	19.0 ± 0.3	25.4 ± 0.3^{bc}	32.6 ± 0.4^{bc}	39.8 ± 0.5^{bc}

[a] Values presented are means ± SE of body weights with 26–41 pups per lead exposure level by seizure condition (treatment cells). The data were analyzed by (1) an overall unweighted means ANOVA with repeated measures on all the data, (2) an unweighted means ANOVA at each age, and (3) multiple pairwise comparisons among treatment cell means at each age by Tukey's HSD test. Significant effects from the overall ANOVA are presented in the text of paper. Significant Pb main effects from ANOVA at each age are indicated on the rows labelled "All Pups" and "All Pups" groups which share the same superscript differed significantly as indicated below. Significant differences between means at each age are indicated on the treatment cell means and groups with a prime superscript notation differed from those groups having the same superscript. Thus f' identifies a treatment cell mean which differed from all other treatment cell groups of the same age labelled with an f. $^{bc}p<0.001$; $^{de}p<0.01$; $^{f}p<0.05$.

animals whose blood and brain Pb concentrations do not differ from those of controls, suggests a permanently altered biochemical or cytoarchitectural substrate. This was reflected in increased latencies in the VER and a decreased ability to follow repetitive flashes [26]. The latter effect indicates that early Pb exposure caused a persistent delay in CNS maturation since it has been shown that the ability of the cerebral cortex to follow repetitive stimulation at increasingly higher frequencies improves with age [22,59].

Pb AND TEMPERATURE REGULATION

Altered environmental temperatures have been shown to influence the susceptibility of animals to Pb poisoning [5, 7, 62, 93]. However, the ontogeny of temperature regulation, an exemplary of integrative functioning, has not been examined following exposure to this toxin. Rats, being poikilothermic at birth, slowly develop the ability to maintain a constant body temperature over a wide range of ambient environmental temperatures [2, 25, 30]. This ability develops during the second postnatal week [2, 25, 30], thereby providing an early developmental period in which to examine the effects of perinatal insult.

This second study, done in collaboration with Dr. R. L. Bornschein, was designed to examine the effects of exposing neonatal rats to Pb using the same exposure level and experimental design as in the first study [26]. From Day 3 to 16 surface body temperature of 24 male control and 18 Pb-exposed rats (one per litter) was determined and recorded. Each pup was removed from the litter, immediately monitored (YSI Telethermometer Model 43, Probe No. 708) on the ventral surface (above heart), and isolated in a 1000 ml polypropylene beaker for 60 min (ambient temperature, 22 ± 1°C). At the completion of this time the surface body temperature was recorded, animals weighed and returned to their home cages.

Initial body temperature (35.7 ± 0.3°C) was the same for both groups of rats. However, following 60 min of isolation there was a significant decrease in the final body temperature of the Pb-exposed neonates compared to controls on Days 3–14 as indicated by an overall ANOVA (Fig. 3). Post-hoc comparison of daily means revealed highly significant differences at all ages examined (Fig. 3).

The ontogenetic pattern of body temperature regulation in controls is comparable to that observed by others [25]. A similar pattern is seen in the Pb-exposed group, however, there is a two to three day maturation lag in the development of the thermoregulatory mechanisms. This is more clearly seen in Fig. 4, which illustrates the degrees lost by each group of pups on each experimental day. In addition to the maturational delay, the slightly altered slope of the developmental curve in the Pb group (Fig. 3) suggests a functional disturbance in this integrative process.

Pb AND MES

Recurrent grand mal seizures occur in approximately 50% of the pediatric population exhibiting the effects of Pb encephalopathy [57, 73, 80]. However, alterations in seizure susceptibility in the presumed symptomatic pediatric population have not been reported. Two reports recently appeared which examined seizure thresholds in adult rodents

TABLE 3

BLOOD AND BRAIN Pb CONCENTRATIONS (μg%) IN Pb-EXPOSED DAMS AND THEIR OFFSPRING

Age (Days)		Pb-Exposed Offspring 10	21	60	Dams 21
No Pb	Blood	2 ± 0	2.2 ± 0.1	3 ± 0	2.4 ± 0.3
	Brain	0.4 ± 0	0.6 ± 0.1	3.2 ± 0.6	–
0.02%	Blood	21.7 ± 1.1	25.2 ± 3.3	2.5 ± 0.3	29.0 ± 3.5
	Brain	6.3 ± 1.0	12.5 ± 1.6	6.9 ± 1.8	–
0.2%	Blood	49.6 ± 4.2	89.4 ± 15.4	5.0 ± 0.5	71.9 ± 11.0
	Brain	18.8 ± 1.3	81.9 ± 23.3	16.3 ± 2.4	–

[a] Values presented are means ± SE with 5–13 animals per Pb treatment at each age. $PbAc_2$ drinking solutions were provided ad lib to dams throughout lactation (Days 0–21), while controls received tap water.

following either acute [1] or chronic developmental [71] Pb exposure. However, neither study examined the effects of Pb exposure on seizure responsiveness during development and in addition, both studies were confounded by the failure to use weight-matched experimental and control animals.

This third study, done in collaboration with Drs. S. R. Overmann and D. E. Woolley, was designed to evaluate changes in seizure development in neonatally Pb-exposed rats by using the MES test [27]. The MES test is an experimental model of grand mal convulsions and an overall indicator of the level of CNS excitation [78,81]. Like the VER, specific components of the MES response emerge in an orderly sequence during development [48,85]. In this study a similar protocol as in the first two studies was used, however, an additional exposure level of Pb was included (0.02% Pb acetate solution to the dam). At ten days of age pups within each litter (3 males, 3 females) were assigned to one of three seizure regimen conditions (No MES, No Strychnine; MES; MES+Strychnine) as shown in Fig. 5. Repeated seizure testing on the same pup with the same treatment was carried out at 10, 12, 14, 16, 18, 20 and 60 (adults) days of age. The total number of neonates studied was 235 from 69 dams while the total number of adults studied was 100. MES seizures were induced by a constant current, 100 μA, sine wave (60 Hz) stimulus delivered for 0.2 sec to the animal's scalp through silver disk electrodes with a stimulus of 100 mA for neonates and 1.0 mA/g body weight for adults [27, 85, 90, 91]. Four electronic timers were activated simultaneously with the shock to the animals and were turned off manually at the end of each phase of the seizure: forelimb flexion, forelimb extension, hindlimb flexion, and hindlimb extension. A stopwatch was used to time postseizure depression, determined as the time to recover righting reflex (RRR). Total tonus was defined as the total duration of the tonic seizure, from its onset to its completion.

Each rat was injected sc with either saline or one of two doses of strychnine sulfate (125 or 500 μg/kg; Merck) 30 min prior to seizure testing. Strychnine sulfate was diluted in physiological saline and given in a volume of 20 μl. Adult rats were not injected prior to seizure testing. The 500 μg/kg strychnine injection dramatically altered the developmental MES pattern described above: all animals, regardless of age or Pb exposure, responded with grade 5 seizures during MES testing. Therefore, except where noted, discussion of results using strychnine administration only refer to the 125 μg/kg dose.

Low-level Pb exposure had no effect on body growth, whereas high-level Pb exposure resulted in a small depression (6–7%) of body growth at 14, 18 and 21 days of age (Table 2). The overall ANOVA on body weight indicated that there was a decrease due to Pb, an increase due to age, and no effect due to MES (or MES+Strychnine) testing. Blood and brain Pb values at weaning in the 0.2% Pb group are similar to those reported in the first study, while values for the 0.02% Pb group are 25.2 ± 3.3 and 12.5 ± 1.6 μg%, respectively (Table 3). Note that at 60 days of age the brain Pb concentrations are still elevated in both Pb groups compared to controls. No sex differences were found at any age with regard to the seizure parameters examined, so all data presented represent the pooled values for males and females.

MES seizure ontogeny in rats is characterized by the sequential appearance of graded seizures of increasing severity: grade 1—clonus; grade 2—tonic florelimb flexion (FF); grade 3—FF followed by tonic forelimb extension (FE); grade 4—FF plus tonic hindlimb flexion (HF) and FE; and grade 5—FF, HF, FE and tonic hindlimb extension (HE).

Neonatally Pb-exposed rates exhibited: (1) earlier ontogenetic appearance of tonic seizures, (2) increased seizure severity during development and (3) long-term disturbances in seizure responsiveness. Low-level strychnine administration during development caused additive interactions with both Pb groups, uncovering subtle effects of toxicant exposure. Alterations in seizure responsiveness were observed in both high and low Pb-exposed rats.

The most sensitive and reliable measure for detecting early maturational differences between No Pb and Pb-treated rats was the percent (Chi-square tests) of animals exhibiting clonus (grade 1) versus the percent exhibiting tonus (grade 2 or greater). In both No Pb and 0.02% Pb-exposed rats approximately 90% responded with clonus on Day 10 while approximately 70% responded with clonus on Day 12. On Day 12 the remainder exhibited tonic seizure grades 2 and 3 (Table 4). In contrast, the 0.2% Pb-exposed rats responded with approximately 25% clonus on Days 10 and 12, while the remainder exhibited tonic seizure grades 2, 3 and 4 (Table 4).

Figures 6 and 7 illustrate the cumulative percentage of neonates exhibiting each seizure phase. For example, a pup

TABLE 4

DISTRIBUTION OF NEONATAL RATS EXHIBITING VARIOUS SEIZURE GRADES DURING MES[a]

Grades		1			2			3			4			5		
Pb Treatment		0	0.02%	0.2%	0	0.02%	0.2%	0	0.02%	0.2%	0	0.02%	0.2%	0	0.02%	0.2%
Age (Days)	Drug Treatment															
10	Saline	**95^{a}**	**90^{b}**	26^{ab}	0^{a}	5^{b}	**47^{ab}**	2^{b}	5^{c}	17^{bc}	0	0	9	2	0	2
	Strychnine	**100^{g}**	**82^{h}**	11^{gh}	0^{g}	14^{h}	**56^{gh}**	0^{g}	0^{h}	33^{gh}	0	5	0	0	0	0
12	Saline	**79^{a}**	**67^{b}**	23^{ab*}	2^{a}	7^{b}	**40^{ab}**	19	24	27	0	2	8	0	0	$2^{\dagger}$
	Strychnine	**87^{g}**	**68^{h}**	0^{gh*}	0^{g}	5^{h}	**39^{gh}**	7	18	**28**	0	0	0	7^{i}	9^{k}	**$33^{ik\dagger}$**
14	Saline	21^{c}	15^{e}	4^{ce}	16	20	17	**49^{*}**	**54**	**$44^{\dagger}$**	12	12	27	2^{*}	0^{*}	8^{*}
	Strychnine	**40^{kg}**	14^{k}	0^{g}	20	19	28	20^{k*}	**38^{g}**	$0^{kg\dagger}$	0^{g}	10	**44^{g}**	20^{*}	19^{*}	28^{*}
16	Saline	0	0	0	0	0	0	**60^{a}**	**$49^{\dagger}$**	**29^{a*}**	18^{cd}	**43^{c}**	**44^{d}**	22^{e}	$8^{ef\dagger}$	$27^{f\dagger}$
	Strychnine	**0**	0	0	0	0	0	**53^{gh}**	$10^{g\dagger}$	6^{h*}	20^{i}	**52^{ij}**	22^{j}	27^{g}	$38^{i\dagger}$	$72^{gi\dagger}$
18	Saline	0	0	0	0	0	0	20	21^{*}	13	10	18	20	**70**	**61**	**67**
	Strychnine	0	0	0	0	0	0	7	0^{*}	0	7	20	11	**87**	**80**	**89**
20	Saline	0	0	0	0	0	0	2	2	4	5	13	4	**93**	**85**	**91**
	Strychnine	0	0	0	0	0	0	0	0	0	0	0	0	**100**	**100**	**100**

[a] Values presented are the percent of neonates exhibiting each seizure grade (see Results section for definition of each grade) during MES with 15–48 animals per Pb treatment by seizure group. For each Pb treatment at each age, bold-faced numbers indicate the MES grade shown by the major proportion(s) of saline or strychnine injected pups. Strychnine (125 μg/kg s.c.) was injected 30 min prior to MES testing. The effects of lead exposure are evaluated statistically by χ^2 analyses of values for pups within the same seizure grade, age, and drug treatment (saline or strychnine); values sharing the same superscript letter differed at the following levels of significance: $^{abgh}p<0.01$; $^{cdij}p<0.05$; $^{efk}0.05<p<0.10$. The effects of strychnine are evaluated statistically by χ^2 analyses of values for pups within the same seizure grade, age, and lead treatment; values sharing the same symbol differed at the following level of significance: $^{*}p<0.05$; $^{\dagger}p<0.01$.

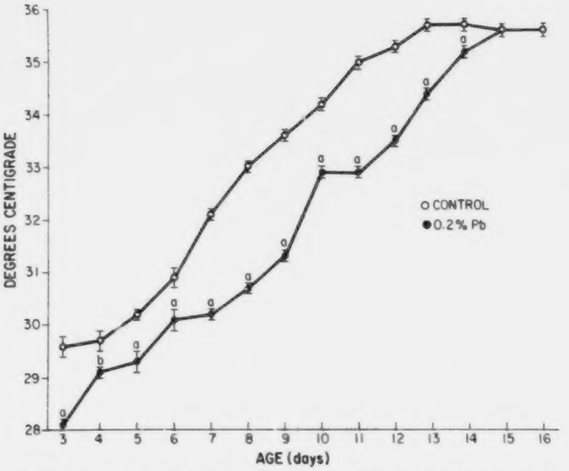

FIG. 3. Final body temperature of control and neonatally Pb-exposed rats following 60 min isolation from dam. Points indicate means ± SE for 18–24 male rats. Initial temperature was the same for both groups, 35.7 ± 0.3°C. Ambient temperature was 22.0 ± 1.0°C. The data were analyzed by an ANOVA and are presented in the text of paper. Post hoc comparison of means within days are indicated by superscript: $p<0.001$ for a; $p<0.02$ for b.

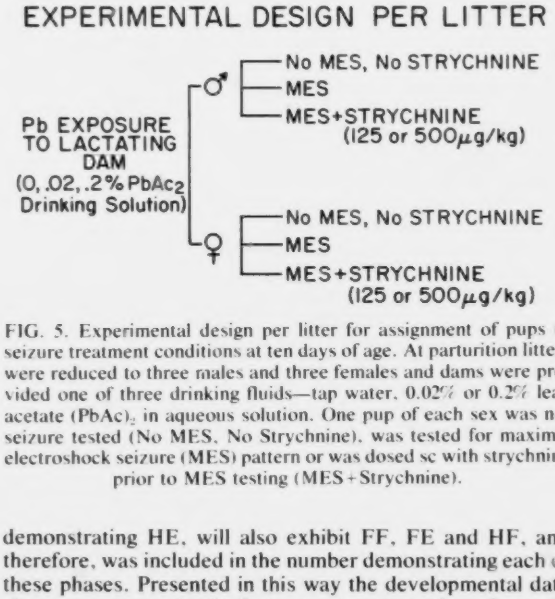
EXPERIMENTAL DESIGN PER LITTER
Pb EXPOSURE TO LACTATING DAM
(0, .02, .2% PbAc2 Drinking Solution)
♂
No MES, No STRYCHNINE
MES
MES+STRYCHNINE (125 or 500µg/kg)
♀
No MES, No STRYCHNINE
MES
MES+STRYCHNINE (125 or 500µg/kg)

FIG. 5. Experimental design per litter for assignment of pups to seizure treatment conditions at ten days of age. At parturition litters were reduced to three males and three females and dams were provided one of three drinking fluids—tap water, 0.02% or 0.2% lead acetate $(PbAc)_2$ in aqueous solution. One pup of each sex was not seizure tested (No MES, No Strychnine), was tested for maximal electroshock seizure (MES) pattern or was dosed sc with strychnine prior to MES testing (MES + Strychnine).

demonstrating HE, will also exhibit FF, FE and HF, and therefore, was included in the number demonstrating each of these phases. Presented in this way the developmental data show even more clearly that a greater proportion of pups in the 0.2% Pb-exposed group exhibited a higher seizure grade than in the other two groups at 10, 12 and 14 days of age.

On Day 12 an additive effect, indicated by a shift in seizure distribution, was observed between the high Pb group and the low-level strychnine injection (Table 4). On Day 16 strychnine administration increased the severity of the seizures by about one seizure grade in both Pb groups. The low Pb group responded almost equally with grade 4 (52%) and 5 (38%) seizures, while the high Pb group exhibited predomi-

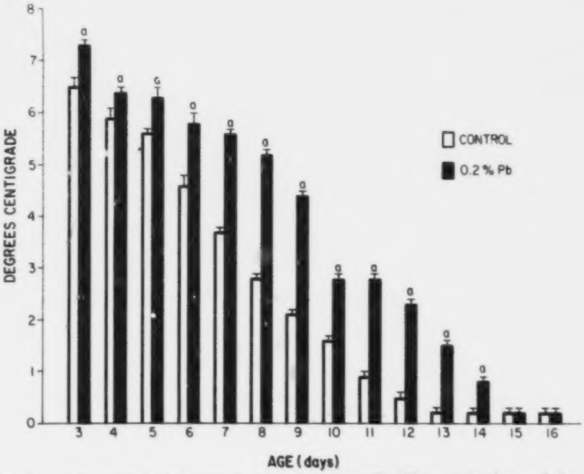

FIG. 4. Degrees lost by control and neonatally Pb-exposed rats following 60 min isolation from dam. Bars indicate mean ± SE for 18–24 male rats. See Fig. 3 for details.

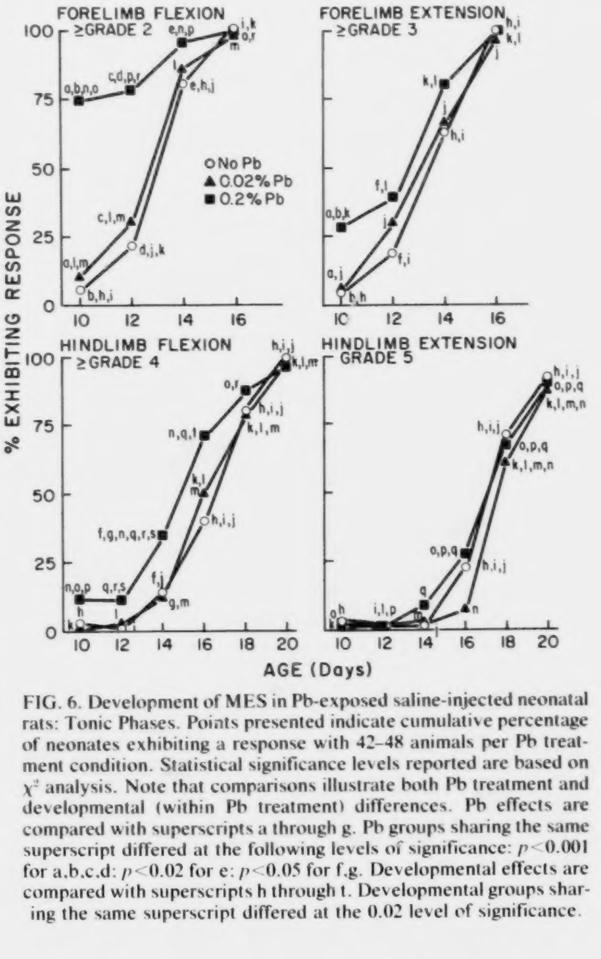

FIG. 6. Development of MES in Pb-exposed saline-injected neonatal rats: Tonic Phases. Points presented indicate cumulative percentage of neonates exhibiting a response with 42–48 animals per Pb treatment condition. Statistical significance levels reported are based on χ^2 analysis. Note that comparisons illustrate both Pb treatment and developmental (within Pb treatment) differences. Pb effects are compared with superscripts a through g. Pb groups sharing the same superscript differed at the following levels of significance: $p<0.001$ for a,b,c,d; $p<0.02$ for e; $p<0.05$ for f,g. Developmental effects are compared with superscripts h through t. Developmental groups sharing the same superscript differed at the 0.02 level of significance.

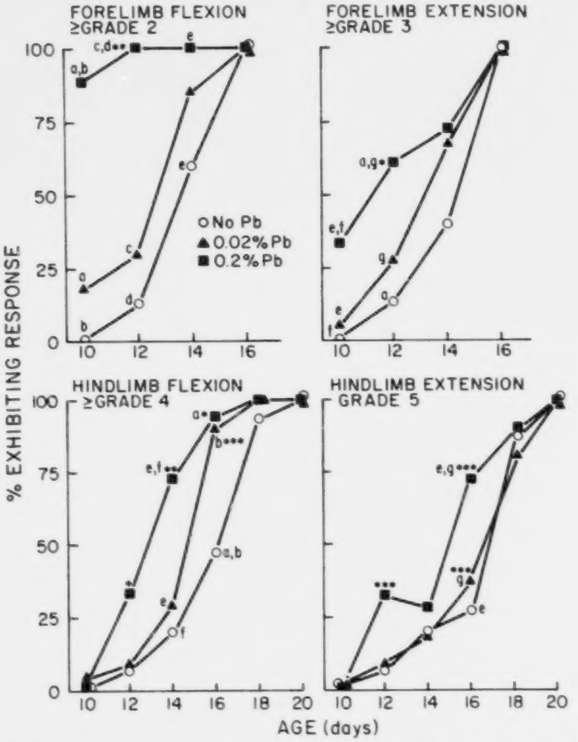

FIG. 7. Development of MES in Pb-exposed strychnine-injected (125 μg/kg) neonatal rats: Tonic Phases. Points presented indicate cumulative percentage of neonates exhibiting a response with 15–22 animals per Pb treatment condition. Statistical significance levels reported are based on χ^2 analysis. Note that comparisons illustrate both Pb treatment and saline versus strychnine injected treatment differences. Pb groups sharing the same superscript differed at the following levels of significance: $p<0.001$ for a,b,c,d: $p<0.02$ for e,f: $p<0.05$ for g. Asterisks indicate difference from similarly aged and Pb-treated saline injected neonatal rats (see Fig. 6) at the following levels of significance: $p<0.005$ for ***: $p<0.02$ for **: $p<0.05$ for *.

nantly grade 5 seizures (72%). By contrast, strychnine-injected No Pb pups exhibited primarily (53%) grade 3 seizures (Table 4).

In the high Pb group, strychnine injection increased the cumulative incidence of FF, FE, HF, and HE at 12 days of age, when compared with saline-injected pups in the same group (Fig. 7). At 14 days of age strychnine treatment compared to saline treatment increased the incidence of HF and HE, but only in the 0.2% Pb group. At 16 days of age strychnine increased the incidence of HF and HE in both Pb groups, but not in the No Pb group (Fig. 7). Thus, at this age, the combination of MES+Strychnine uncovered a subtle effect of 0.02% Pb exposure not detected by the MES test alone.

The additive interactions between Pb exposure and strychnine during maturation may be attributable to Pb-induced alterations in postsynaptic inhibition since strychnine, a drug which removes background inhibition in the spinal cord by blocking postsynaptic inhibition [21,28], at both doses modified the seizure pattern in both Pb groups to a greater degree than in No Pb pups. In addition, the more pronounced effects of strychnine in Pb-exposed rats may reflect alterations in blood-brain-barrier permeability due to Pb toxicity [18, 44, 47, 53, 56] or repeated seizure administration [58] or may reflect alterations in drug-metabolizing capability [3, 4, 14, 24, 68].

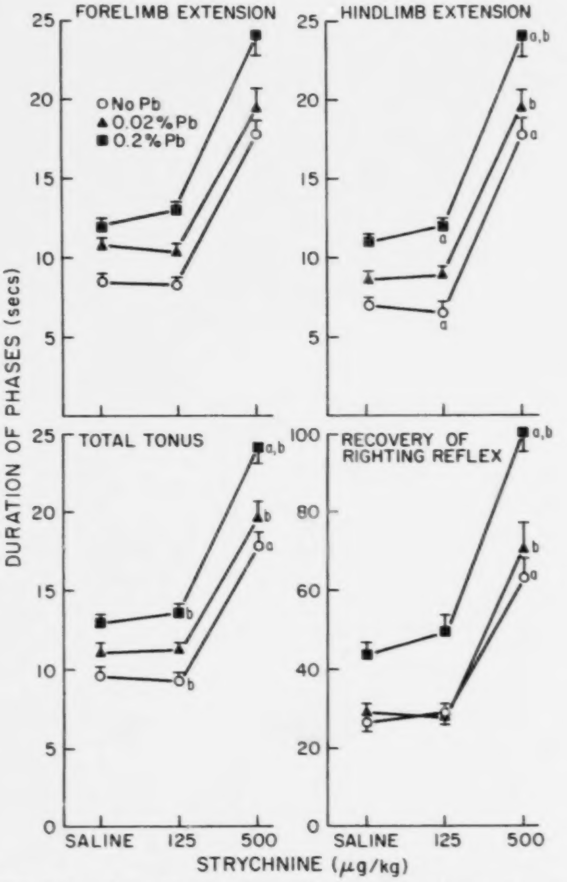

FIG. 8. Durations of forelimb flexion hindlimb extension total tonus and recovery of righting reflex in 20 day old rats. Points presented indicate the means ± SE for 42–48 animals per Pb treatment condition. The data were analyzed by ANOVA and are presented in the text of the paper. Multiple pairwise comparisons among treatment cell means at each strychnine dose are analyzed by Tukey's HSD test. Significant difference between means are indicated by superscripts. Groups sharing same superscript differed from each other: $p<0.01$ for a: $p<0.5$ for b.

As the percent of animals exhibiting each tonic phase of the MES seizure approached 100%, the usefulness of frequency analysis (Chi-square) diminished. Therefore, the durations of the various seizure phases, TT and RRR were analyzed using ANOVA (Table 5) and post-hoc multiple comparisons (Fig. 8). Main Pb effects and main drug effects were noted for FE, HE, TT, and RRR at all ages examined, except RRR on Day 18. Main Pb effects due to the 0.02% group were seen on Day 16 in FE and TT, and on Day 20 in FE, HE, and TT while main drug effects at the low dose of strychnine were only seen on Day 18 in FE and HE. Thus, most of the main Pb effects and main drug effects were due to the 0.2% and 500 μg/kg dose, respectively. ANOVA also

TABLE 5

SUMMARY OF ANALYSIS OF VARIANCE OF DURATIONS OF MES SEIZURE PHASES IN Pb AND STRYCHNINE TREATED NEONATAL RATS

Seizure Phase Examined	Age Studied	Treatment Groups Included in ANOVA Analyses[a] Pb (0, 0.02, 0.2%)	Strychnine (0, 125, 500 μg/kg)	Pb (0, 0.02%)	Strychnine (0, 125 μg/kg)
Forelimb Extension	20	↑↑↑	↑↑↑	↑↑	NS
	18	↑↑↑	↑↑↑	NS	↑
	16	↑↑↑	↑↑↑	↑	NS
Hindlimb Extension	20	↑↑↑	↑↑↑	↑↑	NS
	18	↑↑↑	↑↑↑	NS	↑↑
Total Tonus	20	↑↑↑	↑↑↑	↑↑	NS
	18	↑↑↑	↑↑↑	NS	NS
	16	↑↑↑	↑↑↑	↑	NS
Recovery of Righting Reflex	20	↑↑↑	↑↑↑	NS	NS
	18	↑↑↑	NS	NS	NS
	16	↑↑↑	↑↑↑	NS	NS

[a] Arrows presented indicate results of analyses using an overall unweighted means analysis of variance (ANOVA) with 15–48 animals per Pb treatment by seizure condition. The data analyzed by the ANOVA was partitioned into a 3×3 and then a 2×2 factorial design in order to examine the effects of all Pb and strychnine treatments and the low Pb and low dose strychnine treatment, respectively. Levels of significance as determined by the ANOVA are represented by arrows and are indicated as follows: $p<0.01$ for ↑↑↑; $p<0.02$ for ↑↑; $p<0.05$ for ↑.

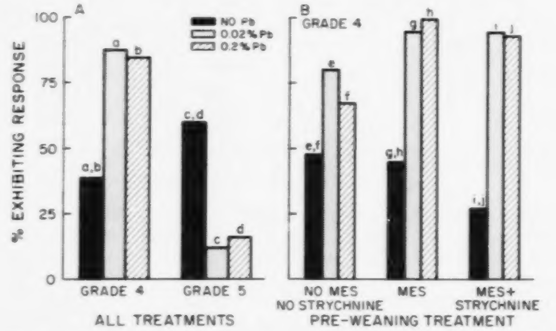

FIG. 9. MES at 60 days of age in neonatally Pb-exposed rats. Bar graphs presented indicate mean percent of animals exhibiting response with 25–42 animals per Pb treatment. Statistical significance levels reported are based on χ^2 analyses. Groups sharing the same superscript differed at the following levels of significance: $p<0.001$ for a,b,c,d,i,j; $p<0.01$ for g,h; $0.05<p<0.10$ for e,f. Part (A) refers to all neonatal treatments combined while (B) separates those rats only exhibiting grade 4 seizures into their individual neonatal seizure-drug treatments.

revealed Pb×drug interactions on Day 16 for FE, TT, and RR (Table 5).

In all three Pb groups, both saline and strychnine-injected, the durations of the flexor (FF and HF) and extensor (FE and HE) components of the MES seizure exhibited an inverse relationship during ontogeny: as the former gradually decreased, the latter gradually increased. At 20 days of age in the Pb-exposed rats compared to No Pb rats, post-hoc analysis revealed that increases in the durations of the MES seizures phases were due to a potentiation between Pb and strychnine (Fig. 8). Both high and low Pb-exposed strychnine-injected groups exhibited longer durations of HE, TT, and RRR than did the No Pb group; however, the low Pb group only showed these change with the 500 μg/kg strychnine pretreatment (Fig. 8).

Previous investigators have established that the longer the duration of extension, the higher is the level of CNS excitation, or the lower is the level of inhibition, and consequently the seizure is more severe [23,81]. The duration data support the fact that throughout development the Pb-exposed rats exhibited more severe seizures. Similar increases in seizure severity, as measured by the MES, have been reported following perinatal exposure to X-irradiation, prenatal manganese deficiency, or postnatal administration of hormones [33, 35, 83, 84, 85].

Following perinatal insult, there is a strong positive correlation in developing rats between the age of appearance of the MES seizure phases and the severity of the seizure [33, 83, 84, 85]. The results of this experiment are consistent with the above findings. The more severe seizures in the Pb groups were accompanied by the earlier ontogenetic appearance of several MES seizure phases (Table 4).

Long-term alterations (60 days of age) in seizure responses were observed in all rats exposed to Pb as neonates, regardless of dose of Pb or other developmental treatments (No MES, No Strychnine: MES: MES+Strychnine) (Fig. 9). No differences in body weight between Pb exposure groups were observed at 60 days of age: male and female mean weight was 291 and 223 g, respectively. Collapsing across preweaning seizure treatments approximately 85% of the Pb-exposed rats exhibited grade 4 seizures compared to only 38% of the No Pb rats (Fig. 9A). In other words, Pb-exposed rats were mostly hindlimb flexors, not hindlimb extensors. A more detailed examination taking into account experimental treatment as a neonate revealed that there was a gradation of statistical significance depending upon the stress of the neonatal treatment. The No MES, No Strychnine group ex-

TABLE 6

AGE AT APPEARANCE OF AUDITORY STARTLE, EYE OPENING AND AIR RIGHTING[a]

	No Pb	0.02% Pb	0.2% Pb	All Pups
Auditory Startle				
No MES, No Strychnine	11.9 ± 0.1^{g}	11.8 ± 0.2	11.7 ± 0.1^{h}	11.8 ± 0.1^{e}
MES	12.2 ± 0.1	12.0 ± 0.2	12.0 ± 0.2	12.1 ± 0.1
MES + Strychnine	12.3 ± 0.1^{g}	12.2 ± 0.2	12.3 ± 0.2^{h}	12.2 ± 0.1^{e}
All Pups	12.1 ± 0.1^{d}	12.0 ± 0.1^{d}	12.0 ± 0.1	
Eye Opening				
No MES, No Strychnine	13.5 ± 0.1^{b}	13.8 ± 0.2^{b}	13.7 ± 0.2	13.7 ± 0.1
MES	13.7 ± 0.1	13.8 ± 0.1	13.9 ± 0.2	13.8 ± 0.1
MES + Strychnine	13.7 ± 0.2	14.1 ± 0.1	13.7 ± 0.2	13.8 ± 0.1
All Pups	13.6 ± 0.1^{bc}	13.9 ± 0.1^{b}	13.8 ± 0.1^{c}	
Air Righting				
No MES, No Strychnine	16.8 ± 0.2^{g}	17.0 ± 0.1	17.3 ± 0.3	17.0 ± 0.1^{ef}
MES	17.2 ± 0.1	17.3 ± 0.1	17.8 ± 0.2	17.4 ± 0.1^{e}
MES + Strychnine	17.3 ± 0.2^{g}	17.3 ± 0.2	17.6 ± 0.2	17.4 ± 0.1^{f}
All Pups	17.1 ± 0.1	17.2 ± 0.1^{c}	17.6 ± 0.1^{c}	

[a] Values presented are means ± SE with 25–38 animals in each Pb exposure by seizure regimen condition. Statistical significance levels reported are based on χ^2 analyses of the number of pups in which landmark first appeared on each day's teating. Presentation of mean ± SE values was chosen to facilitate data reduction. Groups sharing the same superscript differed at the following levels of significance: Pb exposure effects – $^{b}p<0.01$, $^{c}p<0.05$, $^{d}0.10>p>0.05$; Seizure regimen effects – $^{e}p<0.01$, $^{f}p<0.05$, $^{gh}0.10>p>0.05$.

hibited the least significant effect while the MES+Strychnine group exhibited the most significant effect (Fig. 9B).

Analysis of the HF duration data for those rats exhibiting grade 4 seizures revealed no differences between any Pb or preweaning treatment condition. However, the mean HF duration for these flexor rats was 10–11 sec compared to only 3–5 sec mean duration for those rats exhibiting both HF and HE. This suggests that the altered seizure pattern in adults was due to greater inhibition on extensor than on flexor motoneurons. Woolley [91], using DDT or pentobarbital, demonstrated that when the duration of flexion exceeded 5 sec, extension was not likely to occur.

The results of this investigation demonstrated that neonatal Pb exposure in rats produced two temporally-distinct alterations in MES responses: one during preweaning development and the other at 60 days of age. Neonatal rats exposed to Pb exhibited earlier ontogenetic appearance of tonic seizures, increased duration of recovery of righting reflex and increased seizure severity during development. Although small (6–7%) differences in body weight were found between the No Pb and the 0.2% Pb-exposed group during development, these effects were not seen prior to Day 14 and therefore cannot account for the observed alterations in seizure responses in the 0.2% group on Day 10. At 60 days of age, regardless of neonatal seizure treatment, most Pb-exposed rats did not undergo hindlimb extension during the MES, whereas the controls did.

Pb AND REFLEX PATTERNS AND NEUROMUSCULAR PERFORMANCE

Measures of reflex ontogeny and neuromuscular development such as, auditory startle, air righting, eye opening, and performance on negative geoxtaxis and forelimb suspension, provide simple, yet useful, data on the overall sensory-motor competence of the developing neonate. Alterations in these measures have been induced by a variety of perinatal insults [11, 20, 52, 55, 63, 72, 76, 86, 92].

This study, done in collaboration with Drs. Overmann and Woolley, uses the same animals as in the MES study [55]. Age at first appearance of full eye opening (both eyes), auditory startle and air righting as well as testing on the negative geotaxis and forelimb suspension were determined as described previously [31, 55, 92]. From nine to 15 days of age each pup was observed for eye opening and auditory startle while air righting was examined from 15 days of age until the appearance of a criterion response. From four to eight days of age each pup was evaluated on the negative geotaxis, a measure of neuromuscular performance and coordination. On Days 10 to 14 pups were tested on forelimb suspension, a measure of forelimb muscular strength and endurance.

Age of full eye opening was delayed approximately one-half day by both low and high Pb exposure compared to controls, but was unaffected by MES testing. The development of the air-righting reflex was delayed one-half day by the high Pb treatment while MES testing delayed the appearance of this reflex an additional half-day in all seizure-tested rats compared to non-seizure tested rats. Pb exposure had no effect on the first appearance of the auditory startle response whereas MES+Strychnine treatment delayed the onset of this response one-half day in all pups (Table 6).

These results agree with others [63] who found no effect of maternal Pb exposure on age at appearance of the auditory startle response and approximately one-half day delay in full eye opening and appearance of the air-righting reflex in rat offspring of normal body weight. Delayed eye opening was also reported in Pb-exposed mice with markedly impaired body growth [70]. The effect on eye opening in exposed ro-

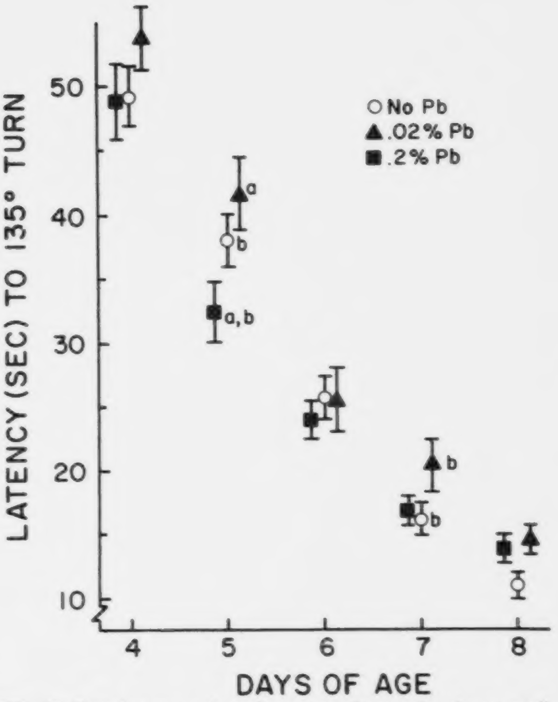

FIG. 10. Negative geotaxis performance of control and postnatally Pb-exposed rats. Points indicate means ± SE with 28–30 performance scores (two scores per litter—one for females and one for males) per data point. Pups were placed head downward on an inclined plane and the latency to a criterion turn up the plane was measured. ANOVA on the groups' scores showed the following relations: No Pb vs 0.02% Pb – $0.10 > p > 0.05$; No Pb vs 0.2% Pb—$p > 0.10$; 0.02% Pb vs 0.2% Pb—$p < 0.05$. For pairwise comparisons within days: $^{a}p < 0.05$ $^{b}0.10 > p > 0.05$.

dents probably reflects Pb-induced delay in normal somatic ontogeny.

Negative geotaxis, a test using gravity as an eliciting stimulus, was conducted by placing rat pups head downward longitudinally aligned on an inclined plane (20° from horizontal). Pups were scored on the direction and latency to turn 135° from the starting line [55]. All pups regardless of treatment showed a developmental decrease in the latency for negative geotaxis-induced turning (Fig. 10). The low Pb-exposed rats exhibited a borderline significant increase in latency (were slower) compared to the other two groups. No differences were found with regard to the proportion of right or left turns between groups. The results from this developmental test are internally consistent with the air-righting reflex data in this study. These data suggest that Pb acts to impair muscular coordination.

In contrast to the above findings, no Pb group differences were found with respect to the forelimb suspension test. A slight effect due to MES testing was found in all Pb treatment groups [55]. These data suggest that Pb exposure does not alter forelimb muscular strength and endurance.

Pb AND SOMATIC INDICES OF DEVELOPMENT

In collaboration with Drs. Overmann and Woolley, hematocrit, brain and organ weight data were obtained from litter mates of rats used in the MES study, providing additional indices of the effects of Pb exposure on somatic development. Hematocrit, organ weight data, water intake and growth measures, were also collected for dams as physiological indicators of the effects of maternal Pb exposure [55].

Dams' drinking water with 0.2% $PbAc_2$ added had decreased fluid consumption by approximately 15% compared to the No Pb and 0.02% groups. Despite this reduced fluid intake, no differences in dam body weights due to Pb exposure were found during the lactation period. Dams' drinking either 0.02% or 0.2% Pb solutions throughout lactation had hypertrophied kidneys, reduced hematocrits, and normal weight adrenals.

Pups, like dams, at weaning in both Pb groups exhibited enlarged kidneys and reduced hematocrits. In addition, pups in 0.2% group had decreased adrenal weights compared to the other two groups. In agreement with Fox *et al.* [26], no differences in whole brain (minus cerebellum) or cerebellum weight were found between groups.

PHYSIOLOGICAL AND BIOCHEMICAL BASIS FOR Pb EFFECTS

Although the physiological and biochemical basis for the developmental and long-term effects of low level Pb exposure are not clear, delays in synaptogenesis [46] and myelin formation [40], alterations in neurotransmitter systems [19], brain electrolytes [29] and amino acid transport [44] have all been reported following neonatal Pb exposure. It is also possible that the interference of Pb in the normal processes of transmitter release, demonstrated in peripheral synapses [15, 39, 45] may permanently alter synaptic function if this disturbance occurs during the early developmental period. Factors which influence the development of the VER, temperature regulation, MES, reflex patterns and neuromuscular performance include synaptogenesis, myelination, maturation of neurotransmitter systems, and metabolic changes in the rat brain during development [10, 34, 36, 43, 49, 50, 51, 59, 60, 65, 67, 82, 85]. Thus, any or all of the above neurotoxic effects of Pb may account for the observed data.

CONCLUSIONS

This battery of developmental sensory-motor tests provides sensitive and quantifiable techniques for assessing developmental and long-term alterations in CNS functioning (in the same animal, except for the VER as conducted herein) following perinatal insult. All of these tests, except the VER, are simple and cost efficient because they can be performed with a minimal amount of: (1) personnel training, (2) equipment cost, and (3) time invested per animal, thereby making them ideal screening tests. In addition, these tests suggest further areas of study, hopefully leading to the underlying mechanism(s) responsible for the deficit.

ACKNOWLEDGEMENTS

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REFERENCES

1. Adler, M. W. and C. H. Adler. Toxicity to heavy metals and relationship to seizure thresholds. *Clin. Pharmac. Ther.* **22:** 774–779, 1977.
2. Adolf, E. F. Ontogeny of physiological regulations in the rat. *Q. Rev. Biol.* **32:** 89–137, 1957.
3. Alvares, A. P., S. Leigh, J. Cohn and A. Kappas. Lead and methyl mercury: effects of acute exposure on cytochrome P-450 and the mixed function oxidase system in the liver. *J. exp. Med.* **135:** 1406–1409, 1972.
4. Alvares, A. P., A. Fischbein, S. Sassa, K. E. Anderson and A. Kappas. Lead intoxication: Effects on cytochrome P-450 mediated hepatic oxidations. *Clin. Pharmac. Ther.* **19:** 183–190, 1976.
5. Baetjer, A. M., S. N. D. Joardar and W. A. McQuary. Effect of environmental temperature and humidity on lead poisoning in animals. *Archs envir. Hlth.* **1:** 463–477, 1960.
6. Baloh, R. W. The effects of chronic increased lead absorption on the nervous system—A review article. *Bull. L. A. Neurol. Soc.* **38:** 91–99, 1973.
7. Blackman, S. S. The lesions of lead encephalopathy in children. *Bull. Johns Hopkins Hosp.* **61:** 1–62, 1937.
8. Bradley, J. E. and R. J. Baumgartner. Subsequent mental development of children with lead encephalopathy as related to type of treatment. *J. Pediatr.* **53:** 311–315, 1958.
9. Brown, D. Neonatal lead exposure in the rat: Decreased learning as a function of age and blood lead concentration. *Toxic. appl. Pharmac.* **32:** 628–637, 1975.
10. Buchanan, A. R. and R. M. Hill. Temperature regulation in albino rats correlated with determinations of myelin density in the hypothalamus. *Proc. Soc. exp. Biol. Med.* **66:** 602–608, 1947.
11. Butcher, R., K. Hawver, K. Kazmaier and W. Scott. Postnatal behavioral effects from prenatal exposure to teratogens. In: *Basic and Therapeutic Aspects of Perinatal Pharmacology*, edited by P. Morselli, S. Garattini and F. Sereni. New York: Raven Press, 1974, pp. 171–176.
12. Byers, R. and E. Lord. Late effects of lead poisoning on mental development. *Am. J. Dis. Child.* **66:** 471–494, 1943.
13. Carson, T., G. Van Gelder, G. Karas and W. Buck. Slowed learning in lambs prenatally exposed to lead. *Archs envir. Hlth.* **29:** 154–156, 1974.
14. Chow, C. P. and H. H. Cornish. Effects of lead on the induction of hepatic microsomal enzymes by phenobarbital and 3,4-benzpyrene. *Toxic. appl. Pharmac.* **43:** 219–228, 1978.
15. Cooper, G. P. and D. Steinberg. Effects of cadmium and lead on adrenergic neuromuscular transmission in the rabbit. *Am. J. Physiol.* **232:** C128–C131, 1977.
16. Coyle, I., M. J. Wayner and G. Singer. Behavioral teratogenesis: A critical evaluation. *Pharmac. Biochem. Behav.* **4:** 191–200, 1976.
17. Dobbing, J. Undernutrition and the developing brain. In: *Developmental Neurobiology*, edited by W. A. Himwich. Springfield, Illinois: C. C. Thomas, 1970, pp. 241–261.
18. Domer, F. R. and J. C. Llera. Blood-brain barrier permeability changes caused by lead exposure and amphetamine in mice. *Res. Communs Psychol. Psychiatr. Behav.* **3:** 101–108, 1978.
19. Dubas, T. C., A. Stevenson, R. L. Singhal and P. D. Hrdina. Regional alterations of brain biogenic amines in young rats following chronic lead exposure. *Toxicology* **9:** 185–190, 1978.
20. Eayrs, J. and W. Lishman. The maturation of behaviour in hypothyroidism and starvation. *Br. J. Anim. Behav.* **3:** 17–24, 1955.
21. Eccles, J. C. Pharmacology of central inhibitory synapses. *Br. Med. Bull.* **21:** 19–25, 1965.
22. Ellingson, R. J. and R. C. Wilcott. Development of evoked responses in visual and auditory cortices of kittens. *J. Neurophysiol.* **23:** 363–375, 1960.
23. Esplin, D. W. and J. W. Freston. Physiological and pharmacological analysis of spinal cord convulsions. *J. Pharmac. exp. Ther.* **130:** 68–80, 1960.
24. Fischbein, A., A. P. Alvares, K. E. Anderson, S. Sassa and A. Kappas. Lead intoxication among demolition workers: The effect of lead on the hepatic cytochrome P-450 system in humans. *J. Toxic. Envir. Hlth.* **3:** 431–437, 1977.
25. Fowler, S. J. and C. Kellogg. Ontogeny of thermoregulatory mechanisms in the rat. *J. comp. physiol. Psychol.* **89:** 738–746, 1975.
26. Fox, D. A., J. P. Lewkowski and G. P. Cooper. Acute and chronic effects of neonatal lead exposure on development of the visual evoked response in rats. *Toxic. appl. Pharmac.* **40:** 449–461, 1977.
27. Fox, D. A., S. R. Overmann and D. E. Woolley. Neurobehavioral ontogeny of neonatally lead exposed rats. II. Maximal electroshock seizures in developing and adult rats. *Neurotoxicology* **1:** 148–169, 1979.
28. Franz, D. N. Central nervous system stimulants. In: *The Pharmacological Basis of Therapeutics*, 5th edition, edited by L. S. Goodman and A. Gilman. New York: Macmillan Publishing Company, 1975, pp. 359–361.
29. Goldstein, G. W. and I. Diamond. Metabolic basis of lead encephalopathy. *Res. Publs Ass. Res. nerv. ment. Dis.* **53:** 293–303, 1974.
30. Hahn, P., O. Koldovský, J. Krecek, J. Martinek and Z. Valek. Endocrine and metabolic aspects of the development of homeothermy in the rat. In: *Somatic Stability in the Newly Born*. Boston: Little, Brown and Company, 1961, pp. 131–155.
31. Hard, E. and K. Larsson. Development of air righting in rats. *Brain Behav. Evolut.* **11:** 53–59, 1975.
32. Hastings, L., G. Cooper, R. Bornschein and I. Michaelson. Behavioral effects of low level neonatal lead exposure. *Pharmac. Biochem. Behav.* **7:** 37–42, 1977.
33. Heim, L. M. and P. S. Timiras. Gonad-brain relationship: Precocious brain maturation after estradiol in rats. *Endocrinology* **72:** 598–606, 1963.
34. Hensel, H. Neural processes in thermoregulation. *Physiol. Rev.* **53:** 948–1017, 1973.
35. Hurley, L. S., D. E. Woolley, F. Rosenthal and P. S. Timiras. Influence of manganese on susceptibility of rats to convulsions. *Am. J. Physiol.* **204:** 493–496, 1963.
36. Jacobsen, S. Sequence of myelinization in the brain of the albino rat. A. Cerebral cortex, thalamus, and related structures. *J. comp. Neurol.* **121:** 5–29, 1963.
37. Jacobson, M. *Developmental Neurobiology*. New York: Plenum Press, 1978.
38. Kostial, K., T. Malijkovic and S. Jugo. Lead acetate toxicity in rats in relation to age and sex. *Archs Toxikol.* **31:** 265–269, 1974.
39. Kober, T. and G. P. Cooper. Lead competitively inhibits calcium-dependent synaptic transmission in the bullfrog sympathetic ganglion. *Nature, London* **262:** 704–705, 1976.
40. Krigman, M. R., M. J. Druse, T. D. Traylor, M. H. Wilson, L. R. Newell and E. L. Hogan. Lead encephalopathy in the developing rat: Effect upon myelination. *J. Neuropath. exp. Neurol.* **33:** 58–73, 1974.
41. Lehotzky, K. and I. Meszaros. Alteration of electroencephalogram and evoked potential in rats induced by organic mercury. *Acta pharmac. tox.* **35:** 180–184, 1974.
42. Lindsley, D. B. The role of nonspecific reticulo-thalamocortical systems in eotion. In: *Physiological Correlates of Emotion*, edited by P. Black. New York: Academic Press, 1970, pp. 147–188.
43. London, E. D., F. J. Vocci and G. Butterbaugh. Age-dependent modification of the maximal electroshock convulsive threshold by central monoamine reduction in rats. *Pharmacologist* **19:** 214, 1977.
44. Lorenzo, A. V. and M. Gewirtz. Inhibition of [^{14}C] tryptophan transport into brain of lead exposed neonatal rabbits. *Brain Res.* **132:** 386–392, 1977.
45. Manalis, R. S. and G. P. Cooper. Presynaptic and postsynaptic effects of lead at the frog neuro-muscular junction. *Nature, London* **243:** 354–356, 1973.

46. McCauley, P. T. and R. J. Bull. Lead-induced delays in synaptogenesis in the rat cerebral cortex. *Fedn Proc.* **37:** 740, 1978.
47. Miller, E., P. Sapienza, T. C. Michel, V. L. Olivito, E. L. Earl and E. J. Van Loon. Some aspects of neurotoxic effects of lead in neonate beagle dogs. *Fedn Proc.* **34:** 287, 1975.
48. Millichap, J. G. Development of seizure patterns in newborn animals. Significance of brain carbonic anhydrase. *Proc. Soc. exp. Biol. Med.* **96:** 125–129, 1957.
49. Mourek, J., W. A. Himwich, J. Myslivecek and D. A. Callison. The role of nutrition in the development of evoked cortical responses in rat. *Brain Res.* **6:** 241–251, 1967.
50. Myers, R. D. Temperature regulation: Neurochemical systems in the hypothalamus. In: *The Hypothalamus*, edited by W. Haymaker, E. Anderson and W. Nauta. Springfield, Illinois: C. C. Thomas, 1969, pp. 506–523.
51. Noback, G. R. and D. P. Purpura. Postnatal ontogenesis of neurons in cat neocortex. *J. comp. Neurol.* **117:** 291–307, 1961.
52. Olson, K. and G. Boush. Decreased learning capacity in rats exposed prenatally and postnatally to low doses of mercury. *Bull. Environ. Contam. Toxic.* **13:** 73–79, 1975.
53. O'Tuama, L. A., C. S. Kim, J. T. Gatzy, M. R. Krigman and P. Mushak. The distribution of inorganic lead in guinea pig brain and neural barrier tissues in control and lead-poisoned animals. *Toxic. appl. Pharmac.* **36:** 1–9, 1976.
54. Overmann, S. Behavioral effects of asymptomatic lead exposure during neonatal development in rats. *Toxic. appl. Pharmac.* **41:** 459–471, 1977.
55. Overmann, S. R., D. A. Fox and D. E. Woolley. Neurobehavioral ontogeny of neonatally lead-exposed rats. I. Reflex development and somatic indices. *Neurotoxicology* **1:** 125–147, 1979.
56. Pentschew, A. and F. Garro. Lead-encephalo-myelopathy of the suckling rat and its implications on the porphyrinopathic nervous diseases. *Acta Neuropath.* **6:** 266–278, 1966.
57. Perlstein, M. A. and R. Attala. Neurologic sequelae of plumbism children. *Clin. Pediatr.* **5:** 292–298, 1966.
58. Petito, C. K., J. A. Schaefer and F. Plum. Ultrastructural characteristics of the brain and blood-brain barrier in experimental seizures. *Brain Res.* **127:** 251–267, 1977.
59. Purpura, D. P. Morphophysiological basis of elementary evoked response patterns in the neocortex of the newxorn cat. *Ann. N.Y. Acad. Sci.* **92:** 840–859, 1961.
60. Purpura, D. P. Ontogenetic models in studies of cortical seizure activities. In: *Experimental Models of Epilepsy*, edited by D. P. Purpura, J. K. Penry, D. M. Woodbury, D. B. Tower and R. D. Walter. New York: Raven Press, 1972, pp. 531–556.
61. Pylkkö, O. O. and D. M. Woodbury. The effect of maturation on chemically-induced seizures in rats. *J. Pharmac. exp. Ther.* **131:** 185–190, 1961.
62. Rapoport, M. and M. Rubin. Lead poisoning: A clinical and experimental study of factors influencing seasonal incidence in children. *Am. J. Dis. Child.* **61:** 245–257, 1941.
63. Reiter, L., G. Anderson, J. Laskey and D. Cahill. Developmental and behavioral changes in the rat during chronic exposure to lead. *Envir. Hlth. Perspect.* **12:** 119–123, 1975.
64. Rodier, P. Behavioral teratology. In: *Handbook of Teratology*, Vol. 4, edited by J. Wilson and F. Fraser. New York: Plenum Publishing Corp., 1978, pp. 397–428.
65. Rose, G. H. and D. B. Lindsley. Visually evoked electrocortical responses in kittens: Development of specific and nonspecific systems. *Science* **148:** 1244–1246, 1965.
66. Salas, S. and S. Schapiro. Hormonal influences upon the maturation of the rat brain's responsiveness to sensory stimuli. *Physiol. Behav.* **5:** 7–11, 1970.
67. Scheibel, M. E. and A. B. Scheibel. Selected structural-functional correlations in postnatal brain. In: *Brain Development and Behavior*, edited by M. D. Sterman, D. J. McGinty and A. M. Adinolfi. New York: Academic Press, 1971, pp. 1–21.
68. Scoppa, P., M. Roumengous and W. Penning. Hepatic drug metabolizing activity in lead-poisoned rats. *Experientia* **29:** 970–972, 1973.
69. Seppalainen, A. M., S. Tola, S. Hernberg and B. Kock. Subclinical neuropathy at "safe" levels of lead exposure. *Archs Envir. Hlth.* **30:** 180–183, 1975.
70. Silbergeld, E. and A. Goldberg. Hyperactivity: A lead-induced behavior disorder. *Envir. Hlth Perspect.* **7:** 227–232, 1974.
71. Silbergeld, E. K., L. P. Miller, S. Kennedy and N. Eng. Lead and seizures: Role of γ-aminobutyric acid. *Soc. Neurosci. Abst.* **3:** 323, 1977.
72. Smart, J. and J. Dobbing. Vulnerability of developing brain. II. Effects of early nutritional deprivation on reflex ontogeny and development of behaviour in the rat. *Brain Res.* **28:** 85–95, 1971.
73. Smith, H. D., R. L. Baehner, T. Carney and W. J. Majors. The sequelae of pica with and without lead poisoning. *Am. J. Dis. Child.* **105:** 609–616, 1963.
74. Sobotka, T., R. Brodie and M. Cook. Psychophysiological effects of early lead exposure. *Toxicology* **5:** 175–191, 1975.
75. Spyker, J. Behavioral teratology and toxicology. In: *Behavioral Toxicology*, edited by B. Weiss and V. G. Laties. New York: Plenum Press, 1975, pp. 311–349.
76. Spyker, J. and D. Avery. Neurobehavioral effects of prenatal exposure to the organophosphate diazinon in mice. *J. Toxic. Envir. Hlth.* **3:** 989–1002, 1977.
77. Sterman, M. B., D. J. McGinty and A. N. Adinoki. *Brain Development and Behavior.* New York: Academic Press, 1971.
78. Swinyard, E. A. Electrically induced convulsions. In: *Experimental Model of Epilepsy*, edited by D. P. Purpura, J. K. Penry, D. M. Woodbury, D. B. Tower and R. D. Walter. New York: Raven Press, 1972, pp. 433–458.
79. Taylor, P. M. Oxygen consumption in new-born rats. *J. Physiol.* **154:** 153–168, 1960.
80. Thurston, D. L., J. N. Middelkamp and E. Mason. The late effects of lead poisoning. *J. Pediatr.* **47:** 413–423, 1955.
81. Toman, J. E. P., E. A. Swinyard and L. S. Goodman. Properties of maximal seizures, and their alteration by anticonvulsant drugs and other agents. *J. Neurophysiol.* **9:** 231–239, 1946.
82. Vernadakis, A. and D. Woodbury. Electrolyte and amino acid changes in rat brain during maturation. *Am. J. Physiol.* **203:** 748–752, 1962.
83. Vernadakis, A. and P. S. Timiras. Effects of whole-body x-irradiation on electroshock seizure responses in developing rats. *Am. J. Physiol.* **205:** 177–180, 1963.
84. Vernadakis, A., J. J. Curry, G. J. Maletta, G. Irvine and P. S. Timiras. Convulsive responses in prenatally irradiated rats. *Expl Neurol.* **16:** 57–64, 1966.
85. Vernadakis, A. and D. M. Woodbury. The developing animal as a model. *Epilepsia* **10:** 163–178, 1969.
86. Wasterlain, C. Effects of neonatal seizures on ontogeny of reflexes and behavior. *Eur. Neurol.* **15:** 9–19, 1977.
87. Werboff, J., I. Goodman, J. Havlena and M. Sikov. Effects of prenatal x-irradiation on motor performance in the rat. *Am. J. Physiol.* **201:** 703–706, 1961.
88. Wiener, G. Varying psychological sequelae of lead ingestion in children. *U.S. Publ. Hlth Rep.* **85:** 19–24, 1970.
89. Wilson, E., J. Bogacz and E. Garcia-Austt. Development of cortical visual evoked responses in the albino rat. *Acta neurol. latinoam.* **12:** 91–105, 1966.
90. Woodbury, L. A. and V. D. Davenport. Design and use of a new electroshock seizure apparatus, and analysis of factors altering seizure threshold and pattern. *Archs int. Pharmacodyn.* **62:** 97–107, 1952.
91. Woolley, D. E. Effects of DDT and of drug-DDT interactions on electroshock seizures in the rat. *Toxic. appl. Pharmac.* **16:** 521–532, 1970.
92. Woolley, D. E. Effects of DDT on the nervous system of the rat. In: *The Biological Impact of Pesticides in the Environment*, edited by J. Gillett. Corvallis, Oregon: Oregon State University Press, 1970, pp. 114–124.
93. Yanase, M. Effects of environmental temperature on chronic lead poisoning. *J. Nagoya City Univ. Med. Ass.* **15:** 55–81, 1964.

Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function
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A Preliminary Test Battery for the Investigation of the Behavioral Teratology of Selected Psychotropic Drugs[1]

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BUTCHER, R. E. AND C. V. VORHEES. *A preliminary test battery for the investigation of the behavioral teratology of selected psychotropic drugs.* NEUROBEHAV. TOXICOL. 1: Suppl. 1, 207–212, 1979.—Pregnant Sprague-Dawley rats received 25 mg/kg of prochlorperazine, 20 mg/kg of fenfluramine, 75 mg/kg of propoxyphene or 200 mg/kg of diazepam daily between the 7th and 20th days of gestation. Vehicle control groups and a positive control group (vitamin A 40,000 IU/kg/day) were similarly prepared. Observations of reproductive performance were made and the offspring examined in a battery of neurobehavioral tests. Fenfluramine and prochlorperazine produced abnormalities in both the reproductive measures and neurobehavioral testing. Propoxyphene produced developmental delays and other signs of "pure" behavioral teratogenesis in that these effects were not anticipated in any of the observations of reproductive performance. Diazepam appeared to have the mildest effect on all the measurements taken. The test methods used in this study appear to be a reasonable initial approach to the development of neurobehavioral screening procedures which are comprehensive, sensitive, and usable.

Behavioral teratology   Prochlorperazine   Fenfluramine   Diazepam   Propoxyphene

INCREASINGLY, a mandate for the inclusion of psychotoxicity studies are included in guidelines for the establishment of the safety of drugs and other chemicals, and instructions for such investigations are part of the drug reproductive guidelines in Britain, France and Japan. Although no comparable requirements exist in the United States the FDA is currently engaged in revising the teratology and reproductive guidelines for new drugs and food additives [13] and this meeting is evidence of the EPA interest in this area.

Our laboratory has had a particular interest in developmental psychotoxicology and for about the past 8 years has examined the post-natal functional consequences of prenatal chemical exposure (behavioral teratology) [4, 6, 7, 8, 9, 10, 12, 16, 23]. Under contract to the FDA, we have also undertaken large scale studies of the behavioral effects from developmental exposure to food additives and psychotropic drugs. As part of this latter effort four psychotropic drugs were examined using a test battery that represents a first attempt to devise a test series that would be usable, sensitive, and comprehensive.

Usability may be defined as the battery's applicability to large scale testing within reasonable cost. Sensitivity must include the conventional indices of reliability and validity and may be operationally defined as the ability of the battery to disclose differences between negative controls and treatments known to produce neurological and behavioral impairments. Comprehensiveness is perhaps the most difficult inasmuch as the range of possible behavioral tests is almost unlimited. At present a limited variety of behavioral functions must be covered under the guidelines of other countries which dictate a minimal level of comprehensiveness. Test protocols of the magnitude suggested by these criteria have only rarely been undertaken heretofore and have not been designed with current guidelines in mind [1,15].

Our experience in investigating the behavioral teratologic potential of selected psychotropic drugs affords an opportunity to evaluate the tentative battery in terms of these criteria and to compare the behavioral results with more usual measures of fertility.

METHOD

Adult Sprague-Dawley rats (Laboratory Supply, Indianapolis, IN) were used for breeding. Females weighed about 260 g at conception, males about 400 g. Date of conception (expelled vaginal plug) was considered Day 0 of gestation (G0) and all females were primiparous. Daily on Days G7–20 females were gavaged with one of the following: 25 mg/kg of prochlorperazine edisylate (Pz) (courtesy of Smith, Kline & French), 20 mg/kg of fenfluramine HCl (Ffl) (courtesy of A. H. Robins), 75 mg/kg of propoxyphene HCl (Pp) (courtesy of Eli Lilly & Co.), 40,000 IU/kg vitamin A palmitate (12 mg/kg) (USV Pharmaceuticals) or saline to the Controls. All drugs were given in solution in saline in a volume of 5 ml/kg except vitamin A (the positive control group), which was solubilized with 12% sorethytan oleate and given in a volume of 1 ml/kg. Doses were based on preliminary dose ranging experiments demonstrating that these doses were not embryotoxic.

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A fourth test drug, diazepam (courtesy of Hoffman-LaRoche), was not water soluble and was, therefore, suspended at a concentration of 10 mg/ml in a solution of 5% acacia. Preliminary studies indicated that diazepam could be administered at 200 mg/kg if the offspring were fostered at birth. This quantity of diazepam was used throughout the entire treatment period and the offspring were, on Days 1–4 of postnatal life, fostered to dams which had received equivalent volumes of acacia during gestation. The diazepam dams were used to rear the acacia offspring in the expectation, based on the pilot investigation, that drug withdrawal would produce disruptions of nurturing behavior. This expectation was not confirmed in subsequent measurements of survival and growth. The cross fostering procedure and the necessity of a separate vehicle (acacia) control group, however, prevent direct comparison to the data from experimental groups using the saline vehicle. The data are, therefore, presented separately in the Results section.

All dams were weighed daily during treatment and all were allowed to litter normally and nurture their offspring. Parturition was considered Postnatal Day 0 (PN0). On Day PN1 all litters were examined externally for malformations, sexed, weighed and any dead fetuses removed. Litters with more than 12 were reduced to 12, balancing for sex. Behavioral testing was conducted on not more than 8 offspring per litter (4 males and 4 females) selected at random on Day PN1 and marked for testing. Testing began on Day PN3 and extended to Day PN70. Offspring were weaned on Day PN21. Due to the amount of time required to conduct some adult tests, only males were examined in spontaneous alternation, Biel maze, active avoidance and passive avoidance tests.

The following number of dams delivered litters in each group: Pz 13 litters, Ffl 14 litters, Pp 10 litters, vitamin A 9 litters, and saline 15 litters. In the diazepam experiment the numbers were diazepam 9 and acacia 8. Five Pz litters were eliminated because they did not meet the requirement of 6 live progeny; one additional litter was eliminated two weeks after birth due to the dams developing an inner ear infection; no Ffl, Pp or vitamin A litters were discarded and only one saline litter was discarded because of too few offspring. In the diazepam experiment one acacia litter was lost due to small litter size, though the dam was still used in the cross fostering procedure and no diazepam litters were discarded. The 2 litter disparity was, for purposes of cross fostering, handled as follows. Although there were only 7 usable acacia litters there were 8 acacia dams, which accommodated 8 of the 9 diazepam litters, the final diazepam litter was fostered to an extra untreated lactating dam that had also just delivered. The 7 acacia litters were cross fostered to their matched diazepam dams and the 2 remaining diazepam dams were eliminated (diazepam and acacia dams were matched for weight and day of conception prior to group assignment).

Behavioral Tests

Surface righting. Observed daily from Day 3. Rats were tested until all test pups righted in < 2 sec on 2 out of 2 trials on a given day [5,24].

Cliff avoidance. Observed daily from Day 6 until each test pup, when placed on an edge with forepaws and nose just over the edge, showed retraction and/or sideward movement away from the edge [5,24].

Swimming development. Observed on Days PN6, 8 and 10 with experienced raters [5, 20, 24]. Test pups were placed in a tank of water (26.7°C) for a period of 5–15 sec and observed for three aspects of swimming: direction, angle in the water (or head position) and use of limbs.

Negative geotaxis. Observed daily on Days PN6–12. Test pups were timed for completing a 180° turn when placed in a head down position on a 25° inclined plywood surface [2].

Preweaning open field. On Days PN15–17 half of the males and half of the females were observed for 3 min/day in a circular open field (dia. 45.7 cm) for number of section entries (the floor was marked into 20 sectors), number of rearings and latency to exit the central start circle [5].

Postweaning open field. Observations were made on 3 consecutive days (PN41–43) for 3 min/day and scored for number of sections entered, pattern of sections entered, latency to begin exploration, number of rearing instances and number of fecal pellets deposited. The open field was circular and 91.4 cm in dia. and twice the scale of the preweaning open field. Animals were those not tested in the preweaning open field [5,24].

Spontaneous alternation. On Day PN45 all males were given 4 alternation trials in a non-reinforced T-maze (stem 45.7 cm, arms 50.8 cm each) and scored for the number of alternations in each pair of trials.

Biel water maze. This task has been described in detail elsewhere [3,11]. Basically the test consists of 2 phases, determination of straight channel swimming speed (5 trials), followed by maze solution (6 trials), recording time and errors. All males were tested on days PN50-53.

Active avoidance. On days PN65-70 half the males were given trials in a wheel turn active avoidance apparatus described in detail elswhere [17]. On each trial a warning white noise came on for 9 sec during which a one-half turn of the wheel terminated the trial and an avoidance was scored; if no turn was made by the end of the 9 sec warning interval, scrambled footshock was delivered to the grid floor and metal walls (0.75 ma) until a wheel turn response was made.

Passive avoidance. Half the male subjects were tested for 3 consecutive days, one day of training and 2 days of retention testing, beginning between days PN55-57, in a 2 chambered passive avoidance apparatus. On each day the animal was placed in the lighted side, the guillotine door raised and the rat allowed to enter the dark side. Entry into the dark side was timed and on the first day resulted in the divider door closing and the onset of a 1 sec, 1.0 mA shock being delivered through a scrambler to the grid floor. On the next two days (retention) no shock was administered when the animal entered the darkened chamber [5,24].

Rotorod. The rod was 11.4 cm in dia. with its surface roughened by a mixture of paint and sand. All rats were given 2 trials/day on two consecutive days between days PN60-65. On each trial the rat was placed on the wheel and the wheel was gradually accelerated until it reached 30 RPM at which point the trial was timed until the rat fell or up to a limit of 3 min [5,24]. Rats were scored for the amount of time on the rod as a percentage of controls and for the percentage of animals in each group reaching the 3 min criterion.

Statistical Analyses

Body weight and most behavioral data were analyzed using unweighted means analyses of variance procedures. Individual group comparisons were made using Newman-Kuels tests [18,19]. Data in proportions (mortality) was analyzed using Fisher's test for uncorrelated proportions [14].

RESULTS AND DISCUSSION

A summary of the effects resulting from prenatal exposure to the water soluble drugs is presented in Table 1. The details of these results will be presented elsewhere. An inspection of these data suggests that with the exception of Pz all the test compounds (including the vitamin A positive control group) had some effect on measures of fertility and offspring weight. Particularly strong effects were observed in the Pz group in which fewer liters with 6 or more pups were produced, the length of gestation and offspring mortality was increased, and the weight of the surviving offspring was reduced at the PN7 weighing.

TABLE 1

SUMMARY OF EFFECTS FROM PRENATAL EXPOSURE TO PSYCHOTROPIC DRUGS*

Measure	Pz	Ffl	Pp	Pos. Cont. Vit. A
No. of litters delivered	0	0	0	0
No. of litters with 6^+ progeny	– –	0	0	0
Length of gestation	+	0	0	0
Offspring mortality	+	+	0	0
Offspring weight				
PN7	–	–	0	–
PN14	0	0	0	–
PN21	0	0	0	–
Surface righting	0	0	0	– –
Cliff avoidance	0	0	0	–
Swimming				
Direction	0	–	0	– –
Angle	0	–	–	– –
Negative geotaxis	+	–	+	– –
Pivoting locomotion	0	(– +)	+	–
Preweaning open field				
Ambulation	0	0	+	0
Rearing	0	0	+	+
Start latency	0	+	–	0

*Symbol code: minus sign = a significant decrease in the dependent measure; plus sign = a significant increase in the dependent measure; zero = no significant differences. One sign = $p<0.05$, 2 signs = $p<0.01$, except for pivoting where the (– +) set of symbols means a significant decrease followed by a significant increase in pivoting time across days of testing.

TABLE 2

SUMMARY OF EFFECTS FROM PRENATAL EXPOSURE TO PSYCHOTROPIC DRUGS POSTWEANING TESTS*

Measure	Pz	Ffl	Pp	Pos. Cont. Vit. A
Postweaning open field				
Ambulation	0	+	+	+
Rearing	–	+	+	+
Start latency	0	0	0	0
Defecation	0	+	0	+
Spontaneous alternation	0	0	0	0
Straight channel swimming speed	0	0	0	– –
Biel water maze errors	0	0	0	0
Wheel turn avoidance	0	0	+	+
Passive avoidance retention	0	0	0	0
Rotorod performance	–	0	–	–
Body weight (PN70)	0	0	0	0

*Symbol use: same as for Table 1

Further inspection of Tables 1 and 2 with emphasis placed on the behavioral data permits some interesting comparisons with the physical measures. The results of the behavioral testing of the Ffl animals are generally consistent with the increased offspring mortality and reduced offspring weights observed in this group. In this case behavioral examination provides confirmation of the functional relevance of the growth retardation and a description of the nature of the developmental delay. The results of testing the Pz offspring, in which fewer differences from controls were observed, suggest that the effects of this compound at this dosage are less remarkable than the observed reproductive abnormalities. A strikingly different conclusion may be drawn from the Pp test results. Here, in contrast to the absence of effects on reproduction, a large increase in behavioral abnormalities was observed. The pattern of departures from saline control values in the Pp animals can be characterized by a slight developmental delay and increased activity/reactivity. At the dose level used in this study these effects were not presaged by the fertility or growth measures and constitute an occurrence of "pure" behavioral teratogenesis.

The females receiving diazepam during gestation were uniformly observed to have a slight to moderate hypotonia during the treatment period which was followed by slight agitation for the 1–3 day period immediately following termination of treatment. The data from the diazepam treated subjects and acacia controls are displayed in Tables 3–5. A comparison by t-test of the results from the acacia and saline vehicle controls revealed a significant ($p<0.05$) difference only in preweaning open field rearing frequency. The diazepam animals were found to weigh significantly less than acacia females at the end of gestation and a marginally significant ($p<0.06$) increase in offspring mortality was observed.

Behaviorally, prenatal diazepam exposure produced a significant delay in swimming development (angle). A significant effect was found in passive avoidance in which the experimental offspring actually withheld the formally punished response longer than acacia treated controls. Similarly, an increased percentage of diazepam offspring were able to attain criterion performance on the rotorod test. Both these latter effects, in the absence of abnormalities in other measures of response inhibition and locomotor coordination, are difficult to interpret until additional data are available.

TABLE 3

DIAZEPAM SUMMARY: REPRODUCTIVE PERFORMANCE AND TESTS OF BEHAVIORAL DEVELOPMENT*

		Treatment		
Measure		Acacia	Diazepam	Sig.
Length of gestation		22.4 ± 0.2 (8)	22.4 ± 0.2 (9)	NS
Litter size		10.4 ± 0.7 (8)	10.7 ± 1.0 (9)	NS
Preweaning mortality		5% (4/74)	13% (12/96)	$p<0.06$
Gestation weight (G19)		361.8 ± 7.9 (8)	330.1 ± 10.1 (9)	$p<0.05$
Lactation weight (L21)		302.0 ± 10.0 (7)	326.3 ± 8.1 (9)	NS
Offspring weight (PN21)	M	36.7 ± 4.0 (7)	37.4 ± 2.8 (9)	NS
	F	35.4 ± 3.9 (7)	36.3 ± 2.3 (9)	NS
Cliff avoidance		9.3 ± 0.4 (7)	9.7 ± 0.5 (9)	NS
Negative geotaxis (PN6)		38.3 ± 5.7 (7)	38.9 ± 3.7 (9)	NS
Surface righting reflex		9.3 ± 0.6 (6)†	9.8 ± 0.5 (9)	NS
Startle reflex		13.4 ± 0.3 (7)	12.8 ± 0.4 (8)†	NS
Swimming development (PN6, 8, 10)				
Direction (PN8)		2.0 ± 0.1 (5)†	1.9 ± 0.1 (8)†	NS
Angle (PN8)		1.5 ± 0.3 (5)†	0.9 ± 0.2 (8)†	NS
Angle (PN10)		2.0 ± 0.2 (7)	1.4 ± 0.1 (9)	$p<0.05$
Limb usage (PN8)		1.0 ± 0.1 (5)†	0.9 ± 0.04 (8)†	NS
Pivoting (PN7, 9, 11)				
Time		17.2 ± 2.7 (7)	15.7 ± 2.2 (9)	NS
No. of 90° turns		5.4 ± 0.7 (7)	5.4 ± 0.7 (9)	NS

*Values represent the mean ± SEM on a per litter basis with the number of litters tested shown in parentheses. Unless shown separately, male and female data have been combined.

†N's are slightly reduced in these cases due to technician's failure to complete test or equipment malfunction.

TABLE 4

DIAZEPAM SUMMARY: TESTS OF ACTIVITY*

		Treatment		
Measure		Acacia	Diazepam	Sig.
Preweaning open field (PN15–17)				
Ambulation		49.5 ± 2.6 (26)	52.9 ± 3.7 (35)	NS
Rearing		4.0 ± 0.9 (26)	3.9 ± 0.4 (35)	NS
Start latency		7.6 ± 1.6 (26)	11.2 ± 2.6 (35)	NS
Postweaning open field (PN40–45)				
Ambulation	M	45.5 ± 4.5 (12)	46.0 ± 2.9 (14)	NS
	F	63.7 ± 6.5 (10)	57.1 ± 3.1 (13)	NS
Rearing	M	9.1 ± 1.4 (12)	9.6 ± 1.3 (14)	NS
	F	12.5 ± 1.9 (10)	9.9 ± 1.1 (13)	NS
Start latency	M	1.2 ± 0.3 (12)	1.8 ± 0.4 (14)	NS
	F	1.0 ± 0.3 (10)	1.9 ± 0.6 (13)	NS
Defecation	M	2.1 ± 0.7 (12)	1.9 ± 0.3 (14)	NS
	F	1.4 ± 0.6 (10)	2.1 ± 0.4 (13)	NS

*Values represent the mean score across all 3 days of testing ± SEM with the number of animals tested shown in parentheses.

TABLE 5

DIAZEPAM SUMMARY: TESTS OF POSTWEANING BEHAVIORAL PERFORMANCE*

Measure		Treatment		Sig.
		Acacia	Diazepam	
Spontaneous alternation (PN45)		71% (52)	69% (56)	NS
Biel maze–straight alley (PN50–53)		0.17 ± 0.01 (27)	0.15 ± 0.01 (32)	NS
Biel maze–errors (PN50–53)		113.4 ± 12.2 (27)	115.4 ± 10.7 (32)	NS
Wheel turn active avoidance (PN65–70)		31.4 ± 1.7 (13)	32.5 ± 1.1 (17)	NS
24h passive avoidance (PN55–60)		102.7 ± 23.6 (11)	156.8 ± 12.8 (15)	$p<0.05$
Rotorod (PN60–65)	M	22% (27)	53% (32)	$p<0.05$
	F	82% (22)	79% (29)	NS

*Values for Biel maze and avoidance tasks represent means ± SEM. For spontaneous alternation values represent the percentage of trials on which the animals alternated. For rotorod the values represent the percentage of animals reaching the 3 min performance criterion. N's shown in parentheses.

Diazepam could be judged among the psychotropic compounds tested to have the lowest potential as a behavioral teratogen. Tervo *et al.* [21], however, have suggested that prenatal exposure to diazepam at much lower doses than those used here produces developmental abnormalities.

The test battery used in these studies reveals a wide spectrum of behavioral outcomes some of which contrast sharply with physical measures of reproduction. We believe these behavioral results, although limited to a single dose, add substantially to the ability to accurately appraise risk in the use of these compounds. Although the battery is not as comprehensive as is required (sensory tests are needed additions), the sensitivity of the battery appears completely adequate to detect the effects of prenatal vitamin A exposure, and in our laboratory, proved to be a usable test system which was applied without undue difficulty.

REFERENCES

1. Almli, C. R. and R. S. Fisher. Infant rats: Sensorimotor ontogeny and effects of substantia nigra distruction. *Brain Res. Bull.* **2:** 425–459, 1977.
2. Altman, J. and K. Sudarshan. Postnatal development of locomotion in the laboratory rat. *Anim. Behav.* **23:** 896–920, 1974.
3. Biel, W. C. Early age differences in the maze performance of the albino rat. *J. genet. Psychol.* **56:** 439–453, 1940.
4. Brunner, R. L., M. S. McLean, C. V. Vorhees and R. E. Butcher. A comparison of behavioral and anatomical measures of hydroxyurea induced abnormalities. *Teratology* **18:** 379–384, 1978.
5. Brunner, R. L., C. V. Vorhees, L. Kinney and R. E. Butcher. Aspartame: Assessment of developmental psychotoxicity in a new artificial sweetner. *Neurobehav. Toxicol.* **1:** 79–86, 1979.
6. Butcher, R. E. Behavioral testing as a method for assessing risk. *Envir. Health Pers.* **18:** 75–78, 1976.
7. Butcher, R. E., R. L. Brunner, T. Roth and C. A. Kimmel. A learning impairment associated with maternal hypervitaminosis-A in rats. *Life Sci.* **11:** 141–145, 1972.
8. Butcher, R. E., K. Hawver, T. Burbacher and W. Scott. Behavioral effects from antenatal exposure to teratogens. In: *Aberrant Development in Infancy*, edited by N. Ellis. Potomac, Maryland: Erlbaum Assoc., 1975, pp. 161–167.
9. Butcher, R. E., K. Hawver, K. Kazmaier and W. Scott. Postnatal behavioral effects form prenatal exposure to teratogens. In: *Basic and Therapeutic Aspects of Perinatal Pharmacology*, edited by P. L. Morselli, S. Gerattini and F. Serini. New York: Raven Press, 1975, pp. 171–176.
10. Butcher, R. E., W. J. Scott, K. Kazmaier and E. J. Ritter. Post-natal effects in rats of prenatal exposure to hydroxyurea. *Teratology* **7:** 161–165, 1973.
11. Butcher, R., C. Vorhees and H. Berry. A learning impairment associated with induced phenylketonuria. *Life Sci.* **9:** 1261–1268, 1970.
12. Butcher, R. E., C. V. Vorhees and C. A. Kimmel. A learning impairment from maternal salicylate treatment in rats. *Nature New Biol.* **236:** 211–212, 1972.
13. Collins, T. F. X. Reproduction and teratology guidelines: Review of deliberations by the national toxicology advisory committee's reproduction panel. *J. envir. pathol. Toxicol.* **2:** 141–147, 1978.
14. Guilford, J. P. *Fundamental Statistics in Psychology and Education.* New York: McGraw-Hill, 1965.
15. Irwin, S. Comprehensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. *Psychopharmacologia* **13:** 222–257, 1968.
16. Kimmel, C. A., R. E. Butcher, C. V. Vorhees and H. J. Schumacher. Metal-salt potentiation of salicylate-induced teratogenesis and behavioral changes in rats. *Teratology* **10:** 293–300, 1974.
17. Kinney, L. and C. V. Vorhees. A comparison of methylphenidate induced active avoidance and water maze performance facilitation. *Pharmac. Biochem. Behav.* **10:** 437–439, 1979.
18. Kirk, R. E. *Experimental Design: Procedures for the Behavioral Sciences.* Belmont, Calif.: Brooks Cole Publishing, 1968.
19. Kramer, C. Y. Extension of multiple range tests to group means with unequal numbers of replications. *Biometrics* **12:** 307–310, 1956.
20. Schapiro, S., M. Salas and K. Vukovich. Hormonal effects on ontogeny of swimming ability in the rat: Assessment of central nervous system development. *Science* **168:** 147–151, 1979.

21. Tervo, D., C. Kellogg, A. Miller and J. Ison. Prenatal diazepam exposure in rats: Effects on growth and behavioral development of the offspring. *Soc. Neurosci.* **4:** 128, 1978 (Abstract).
22. Vorhees, C. V. Some behavioral effects of maternal hypervitaminosis A in rats. *Teratology* **10:** 269–273, 1974.
23. Vorhees, C. V., R. L. Brunner, C. R. McDaniel and R. E. Butcher. The relationship of gestational age to vitamin A induced post-natal dysfunction. *Teratology* **17:** 271–276, 1978.
24. Vorhees, C. V., R. E. Butcher, R. L. Brunner and T. J. Sobotka. A developmental test battery for neurobehavioral toxicity in rats: A preliminary analysis using MSG, calcium carrageenan and hydroxyurea. *Toxic. appl. Pharmac.* in press, 1979.

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Assays for Behavioral Toxicity: A Strategy for the Environmental Protection Agency[1,2]

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WEISS, B. AND V. G. LATIES. *Assays for behavioral toxicity: A strategy for the environmental protection agency.* NEUROBEHAV. TOXICOL. **1**: Suppl. 1, 213–215, 1979.—Broad agreement on specific approaches or standardized test batteries for assessing behavioral toxicity is unlikely to emerge in the foreseeable future. EPA should reject test standardization in any case, however; standardization stifles progress and, in addition, may bypass unique properties of new types of substances. The optimal strategy is to prescribe a set of functions, such as sensory, motor, and complex performance processes, leaving it to the manufacturer to select adequate tasks. Adequacy would be judged by EPA staff, in consultation with advisory panels, and resolved, in most cases, by a dialogue with the manufacturer.

Behavioral toxicity   Standardization of tests   Screening strategies, behavior

THIS collection of papers provides the most emphatic statement so far of how essential it is for the Environmental Protection Agency to shun test standardization. No obvious approach or battery of behavioral tests emerges as universally suitable for toxicity screening. Too many choices are available for any subset to elicit wide agreement, a reflection of behavioral toxicology's youth and vigor. Furthermore, although it is being called upon to provide simple screening methods, we really need a strategy aimed at the total assessment of risks, one that starts in the laboratory but concludes with monitoring of human populations once a chemical is marketed. The broad choice of assessment methods offered by behavioral toxicology, however, is not a situation to bemoan. It reflects not chaos, but flexibility. Such flexibility commands a price, however. It cannot be purchased without a perceptive review of the strengths and suitability of the tools selected for any particular evaluation. How these are to be weighed was the salient theme of this conference.

A behavioral analog of the Ames test, the bacterial mutagenic assay, is an impossible dream. Mutagenesis is triggered by a limited, if not a single, collection of mechanisms. Behavior, in contrast, is the most diverse of all biological functions, and is subserved by an extraordinary range of mechanisms. The best we can expect from any testing scheme is a restricted sampling of mechanisms, perhaps amplified selectively by a sequential narrowing of specific questions emerging during assessment.

Several current proposals for screening envisage test batteries is made up of relatively simple elements. The absence of complex measures and detailed analysis is assumed to be compensated for by large numbers of animals and the broad scope of the tests. Such batteries can satisfy only a first stage assessment. First stage must be emphasized. Behavioral scientists have turned to complex procedures and instrumentation for reasons surpassing a fascination with slick gadgetry and theoretical minutiae. They have done so because most easy questions already have been answered. Further, behavioral toxicology cannot emphasize only simple questions. Its responsibility is to demonstrate that particular behavioral deficits do not occur under particular circumstances; to do so it must meet standards of experimental rigor and test reproducibility that do not come cheap.

An intermediate strategy would limit the number of different situations to be evaluated and analyze one, or a few, in great detail. For example, one can build on the massive empirical foundation of operant technology and study different behaviors in the same situation. An animal trained to make a particular response in the presence of one set of environmental stimuli, for example, might be trained in the same situation to emit another kind of response to another set of stimuli. Such an approach might achieve operational

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simplicity without sacrificing functional and conceptual complexity. It has worked successfully in behavioral pharmacology, and its reliance on automation reduces many of the problems of reliability afflicting the more conventional batteries.

Psychophysical approaches designed to achieve a finely-tuned assessment of sensory capacity are characterized by both complexity and specificity, but may require extended training and expensive instrumentation. Although they might not be feasible for early screening, such techniques play a significant role in behavioral toxicology. First, they represent the culmination of a sequential screening strategy whose final steps would consist of specifying the precise impact of an agent on various sensory systems. Second, they can tell us which functions and at which parameter values the toxicity of an agent is first expressed, providing cues for human monitoring and guides for ancillary criteria such as morphology and biochemistry. Third, they provide the basis for validating simpler and less comprehensive assessment techniques.

There is no inherent conflict among the various screening strategies. They are all essential. They simply play different roles, all of which are required to fulfill EPA's responsibility. They usually are called upon at different stages of evaluation.

No technique, no combination of techniques, no single subdiscipline will achieve widespread adoption, however, unless it is accompanied by demonstrations of sensitivity and selectivity. Any approach must show responsiveness to relatively small amounts of a chemical and respond differently to different chemicals. One way to develop such characteristics is by designing methods directed at specific classes of agents. If a test battery is aimed at characterizing the sequelae of a well-studied agent such a acrylamide, measures of impairment can be refined to provide a well-focused image of acrylamide toxicity. Such reference substances help us to hone and maintain our tools. Although we have almost no alternatives to this approach, we still remain somewhat uncertain, however, of our tools' predictive power. It is as though we had devised a new test of intelligence, then determined its validity by correlating it with the Stanford-Binet instead of an independent criterion.

One source of independent criteria is coordinate data from morphology, biochemistry, physiology, and pharmacokinetics. If, for example, a portion of the cerebral cortex known to subserve vision is damaged by an agent such as methylmercury, and vision testing indicates correlated deficits in function, we feel confident in our choice of visual tests. But we rarely enjoy such a luxury. We already know that the central nervous system possesses such a huge functional reserve that major damage may be inflicted before any overt impairment appears; "silent damage" is the term used in the methylmercury literature. Such discrepancies are exaggerated by behavioral and neural compensatory mechanisms. Conversely, what independent verification can we extract about agents that leave no easily verifiable traces? Not all behaviorally active agents produce nervous system pathology or enduring neurochemical changes. The mechanisms of mercury vapor toxicity are unknown, with no morphological or chemical guides. Yet, its advanced stages are marked by neurological impairment such as tremor, and its earliest stages by characteristic psychological complaints. Furthermore, the central nervous system may not be the target organ at all in some intoxications. Early, non-specific symptoms may arise from damage to other organ systems. Kidney and liver disease may lead to nervous system dysfunction by indirect routes. The inhibition of peristalsis in the pigeon crop by lead demonstrates how totally unexpected mechanisms may underlie a change in behavior. Last, an agent may well exert a nearly simultaneous affect on morphology and behavior that are not at all related. No one can assume that biological questions always stimulate straightforward answers.

The predictive power of a test, or system of tests, emerges also as a statistical issue. It is most vividly illustrated by the problems of behavioral teratology. Even potent teratogens may damage selectively only small proportions of offspring, which is why postnatal test batteries typically include many different indices. Equivalent problems, perhaps even magnified because dose-related manipulations are difficult to perform in that context, prevail in human studies. So far, most attempts to validate test systems have confined themselves to individual tests and group statistics, noting, whether test "X", say, differentiates between groups of treated and untreated animals. When many different tests and measures are available, however, multivariate statistical techniques enable investigators to express an entire array of findings in a compact format of combined indices that may stimulate the design of new techniques. Nor should behavioral toxicology rely solely on parametric statistics. If only a few animals in a large group respond adversely to a toxic agent, such measures may inadequately reflect the impact of the agent. The distribution of responses in a group represents important data and should not be buried in group calculations. Adequate statistical techniques are available for such analyses as well.

This discussion has emphasized problems and inconsistencies. Can the Environmental Protection Agency, faced with such quandaries, carry out its responsibilities under the Toxic Substances Control Act and other legislation? This conference confirms that it can, and also indicates the strategies to adopt in doing so.

EPA should begin by rejecting any pressure to proclaim standardized tests. Freezing a test battery into regulatory practice is helpful only to underemployed lawyers, because it fosters debate about the minor details of regulatory language, rather than about scientific content. But without explicit descriptions of particular procedures, how is it possible to achieve enough uniformity for regulatory consistency? One way is to follow the examples of Japan and Britain, both of which now require behavioral toxicology for new drugs. Neither nation specifices guidelines or precise tests. Instead, the adequacy of behavioral testing is determined in a dialogue between manufacturer and regulator. EPA's Office of Toxic Substances, however, because of U.S. regulatory traditions and practices, is forced to specify more concretely what it considers to be adequate data. It should do so by adopting a functional approach, that is, by specifying those aspects of behavior it considers critical in evaluating toxicity.

Although they may express divergent opinions on many issues, most practitioners of behavioral toxicology, like those at this conference, agree on certain principles. They agree that both sensory and motor function should be evaluated. They agree that more complex behaviors, such as discriminative and learning processes need to be included in most assessments. They agree that postnatal evaluation cannot stop with early reflex measures, but must include performance after maturation. They agree that reproductive behavior processes are crucial indices of toxicity. EPA strategy, then, would be to list a set of functions accompanied by possible assessment methods for each function. It would be made clear that such methods do not exclude additional, perhaps newer and more sensitive methods, especially since academic, industrial, and government scientists now are engaged in such active pursuit of better methods that it would be surprising if many did not soon emerge.

We envisage a scenario, then, in which a manufacturer brings to EPA a portfolio of results on behavioral tests with a new agent. The choice of tests reflects the sponsor's opinion about the most likely effects of concern. EPA staff then reviews the portfolio, exercising professional judgment about the adequacy of the techniques and the data. They take into account the background of the manufacturer's scientists, the scientific history of the chosen methods, data treatment, reproducibility, comparative data from reference substances, and perhaps other criteria upon which, say, a referee for a journal might base a recommendation for acceptance, revision, or rejection of a manuscript. EPA staff would also exploit the advice of review panels and individual scientists with special qualifications. Should EPA's conclusions conflict with those of the manufacturer, we foresee that most such cases will be resolved in a dialogue rather than in court, provided that the science itself is not blatantly inadequate.

Both the public, represented by EPA, and the manufacturer would gain from such an arrangement. Rather than being locked into obsolete, often inappropriate standards, testing methods could evolve in parallel with the scientific development of the discipline, and the accretion of other toxicologic knowledge. Rather than squandering resources and talent on standardized tests unsuitable for a particular question, behavioral toxicology could then liberate its extraordinary potential to ask more adequate, exacting, and specific questions to everyone's benefit.

Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function
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Final Comments

When I helped spawn this Workshop, I realized that the reward was problematical. When Peter Spencer stated that ours is the first interdisciplinary meeting of a neurotoxicity group, a reward is in sight if this Workshop encourages pursuit of an activity analogous to psychopharmacology—psychotoxicology.

Obviously, it would be presumptuous of me to attempt literally to summarize this symposium. I can recall the questions and issues raised by Norbert Page in his introductory remarks and note how close we came to answering and clarifying them. The convening of this Workshop is a result of a Toxic Substances Control Act requirement to develop test data for behavioral disorders. Since neither behavior nor behavioral is defined in the law, its administrators will do so.

It was my intention that an authoritative group expert in experimental investigation of behavior have a cohesive set of recommendations before the regulatory groups adopt less scientific guides in defining behavior. I encountered an analogous situation shortly after I joined the Environmental Protection Agency. I quickly discovered that the regulators had redefined hydrocarbons to include compounds that no organic chemists would consider as hydrocarbons. The result is that the lesion described by pathologists as proliferative hydrocarbon glomerulonephritis is frequently caused by ketones, alcohols and other nonhydrocarbon compounds. I assumed that something similar would happen to "behavioral" when regulators defined it without adequate input from some group such as this one.

A second incentive was my experience before a Congressional oversight committee on the Michigan incident with polybrominated biphenyls and at a National Institutes of Health meeting on the behavioral effects of these compounds. On both occasions the neurological assessment of behavioral effects was based considerably on anecdotes. The signs and symptoms could not be clearly differentiated from possible effects on endocrine organs.

Page's first question was directed to strengthening routine general toxicity tests to provide more sensitive indicators for carrying out tests for behavioral and neural toxicity. Such indicators are derived from simple observation of the dosed animals and scoring the effects. The outcome determines whether behavioral or neurological tests are necessary. Papers presented at this meeting allow for such an approach. However, it is not foolproof and will miss neurological or behavioral effects in some instances. For instance, promazine was screened in the antimalarial program. The neurological effects, which are obvious, were missed by the observer. The useful effects of the promazines were discovered 13 years later.

Should we require much neuropathology, neurochemistry and neurophysiology measurement during routine toxicity testing? This question will be resolved only after much debate. Two extreme views are current. The pathology oriented investigator of behavior is not convinced of a true effect unless he can demonstrate a true lesion in the integrity of neuronal tissue. The other extreme of this concept is represented by those behaviorists who contend that electrophysiology and lesion placement cannot contribute significantly to an understanding of behavior. The resolution of this question for our purposes will probably be based on an intermediate position.

What existing tests for sensory, motor or cognitive effects are well enough developed and validated so that they can be proposed as standards? Several elegant methods for outwitting animals or for preventing the animals from outwitting the operator were presented at this Workshop. They were presented on the assumption that these methods could be used as tests for predicting behavioral toxicity in humans. For which of these methods is the assumption valid? Much of the discussion of these methods was centered on test details as though this assumption is justified.

What areas require priority for further research on test methods? It became obvious during the discussions that many such areas exist. One such area is further investigation of correlates of behavior. An example of incomplete understanding correlates to behavior was furnished by return of behavior to "normal" in the presence of persistent neuropathology. Failure of correlates of a phenomenon to predict an event is not limited to behavioral science. I am not discouraged by such failures: they have been resolved in other areas.

How well have we met the point raised by Dr. Page? I feel that some have been met, others not. What is needed are tests that will reliably detect neurological effects and behavioral effects of chemicals.

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Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function
Neurobehavioral Toxicology, Vol. 1, Suppl. 1, p. 219. ANKHO International Inc., 1979.

AUTHOR INDEX

ANDO, K. 45

BARBEAU, A. 175
BAUTISTA, S. 113
BIGNAMI, G. 179
BRADFORD, L. D. 73
BRADY, J. V. 73
BUTCHER, R. E. 207

CABE, P. A. 137
COLOTLA, V. A. 113
CORY-SLECHTA, D. A. 129
COX, C. 149

DEWS, P. B. 119

ELSNER, J. 163

FLYNN, E. R. 93
FLYNN, J. C. 93
FOX, D. A. 193

GAUSE, E. 9
GELLER, I. 7, 9
GOULD, D. H. 1

HÄNNINEN, H. 157
HARTMANN, R. J. 9
HIENZ, R. D. 73

JOLICOEUR, F. B. 175

LATIES, V. G. 129, 213
LOOSER, R. 163
LORENZANA-JIMÉNEZ, M. 113
MACPHAIL, R. C. 53
MARGEN, S. 149
MAURISSEN, J. P. J. 23
MCMILLAN, D. E. 105
MERIGAN, W. H. 15
MITCHELL, C. L. 137
MOODY, D. B. 33

PAGE, N. P. 3
PATTON, J. H. 93

REITER, L. W. 53
RICE, D. C. 85
RODRIGUEZ, R. 113
RONDEAU, D. B. 175

SCHAUMBURG, H. H. 187
SEIFTER, J. 9, 217
SPENCER, P. S. 189
STEBBINS, W. C. 7, 33

TAKADA, K. 45
TILSON, H. A. 137

VORHEES, C. V. 207

WAYNER, M. J. 175
WEISS, B. 149, 213
WENGER, G. R. 119
WILLIAMS, J. H. 149
WOOD, R. W. 67

YOUNG, M. 149

ZBINDEN, G. 163

Preceding page blank

Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function
Neurobehavioral Toxicology, Vol. 1, Suppl. 1, pp. 221–225. ANKHO International Inc., 1979.

SUBJECT INDEX

3-Acetyl pyridine
  comparison of neurobehavioral effects induced by various experimental models of ataxia, 175
Acquisition
  behavioral toxicity, 105
  performance, 105
  serial position sequences, 105
Activity
  operant conditioning of infant monkeys for toxicity testing, 85
  quantitative analysis of rat behavior patterns in a residential maze, 163
Affective behavior
  behavioral toxicology, 93
  effects of pre- and post-natal lead exposure, 93
  learning, 93
  social interaction, 93
Ammonia
  inhaled substances, 67
  nitrous oxide, 67
  reinforcing properties, 67
  toluene, 67
Amobarbital
  behavioral assessment of risk-taking and psychophysical function, 73
  behavioral toxicology, 73
Amphetamine
  methodological problems in the analysis of behavioral tolerance, 179
  methods in behavioral toxicology, 179
Antimuscarines
  methodological problems in the analysis of behavioral tolerance, 179
  methods in behavioral toxicology, 179
Applied behavior analysis
  behavioral epidemiology, 149
  food additives, 149
  food colors, 149
  randomization tests, 149
  time series, 149
Ataxia
  3-acetyl pyridine, 175
  comparison of neurobehavioral effects induced by various experimental models, 175
  pyrithiamine, 175
  thiamine deficiency, 175
Atropine
  behavioral toxicity, 119
  caffeine, 119
  schedule-controlled responding, 119
  spontaneous motor activity, 119
  testing for behavioral effects of agents, 119
Auditory discrimination
  carbon monoxide, 9
  discrimination behavior, 9
  environmental contaminants, 9
  ketones, 9
  polybromide biphenyl, 9
Auditory threshold
  behavioral assessment of risk-taking and psychophysical function, 73
  drug-affected sensory threshold changes, 45
  kanamycin, 45
  LSD-25, 45
  pilocarpine, 45
  quinidine, 45
  trailwise tracking method, 45
  visual threshold, 45
Axonopathy
  cellular responses to neurotoxic compounds of environmental significance, 189

Behavioral development
  physiological and neurobehavioral alterations during development in lead exposed rats, 193
Behavioral effects
  psychological test methods: sensitivity to long term chemical exposure at work, 157
Behavioral epidemiology
  applied behavior analysis, 149
  food additives, 149
  food colors, 149
  randomization tests, 149
  time series, 149
Behavioral teratology
  diazepam, 207
  fenfluramine, 207
  preliminary test battery for the investigation of selected psychotropic drugs, 207
  prochlorperazine, 207
  propoxyphene, 207
Behavioral tolerance
  amphetamine, 179
  antimuscarinics, 179
  cannabis derivatives, 179
  CNS depressants, 179
  methodological problems in the analysis of behavioral tolerance, 179
  methods in behavioral toxicology, 179
Behavioral toxicity
  acquisition, 105
  atropine, 119
  caffeine, 119
  performance, 105
  schedule-controlled responding, 119
  screening strategies for the Environmental Protection Agency, 213
  serial position sequences, 105
  spontaneous motor activity, 119
  standardization of tests, 213
  testing for behavioral effects of agents, 119
Behavioral toxicology
  affective behavior, 93
  amobarbital, 73
  amphetamine, 179
  antimuscarinics, 179
  behavioral assessment of risk-taking and psychophysical function, 73
  cannabis derivatives, 179
  chlordiazepoxide, 73
  CNS depressants, 179
  crop dysfunction, 129
  diazepam, 73
  d-methylamphetamine, 73
  effects of pre- and post-natal lead exposure, 93

Preceding page blank

  ethanol, 129
  lead, 129
  learning, 93
  methodological problems in the analysis of behavioral tolerance, 179
  methods, 179
  methylmercury, 129
  physiological and neurobehavioral alterations during development in lead exposed rats, 193
  quantitative analysis of rat behavior patterns in a residential maze, 163
  social interaction, 93

Caffeine
  atropine, 119
  behavioral toxicity, 119
  schedule-controlled responding, 119
  spontaneous motor activity, 119
  testing for behavioral effects of agents, 119
Cannabis derivatives
  methodological problems in the analysis of behavioral tolerance, 179
  methods in behavioral toxicology, 179
Carbon disulfide
  psychological test methods: sensitivity to long term chemical exposure at work, 157
Carbon monoxide
  auditory discrimination, 9
  discrimination behavior, 9
  environmental contaminants, 9
Chlordiazepoxide
  behavioral assessment of risk-taking and psychophysical function, 73
  behavioral toxicology, 73
Chronic inhalation
  effects of solvents on schedule-controlled behavior, 113
CNS depressants
  methodological problems in the analysis of behavioral tolerance, 179
  methods in behavioral toxicology, 179
Cognitive tests
  psychological test methods: sensitivity to long term chemical exposure at work, 157
Comparative behavioral toxicology
  hearing loss, 33
  toxic effects on sensory systems in experimental animal models, 33
Crop dysfunction
  behavioral toxicology, 129
  ethanol, 129
  lead, 129
  methylmercury, 129

Diazepam
  behavioral assessment of risk-taking and psychophysical function, 73
  behavioral toxicology, 73
  preliminary test battery for the investigation of the behavioral teratology of selected psychotropic drugs, 207
Discrimination behavior
  auditory behavior, 9
  carbon monoxide, 9
  environmental contaminants, 9
  ketones, 9
  polybromide biphenyl, 9
Discrimination reversal
  operant conditioning of infant monkeys for toxicity testing, 85
Drug
  3-acetyl pyridine, 175
  ammonia, 67
  amobarbital, 73
  amphetamine, 179
  antimuscarinics, 179
  atropine, 119
  caffeine, 119
  cannabis derivatives, 179
  carbon disulfide, 157
  carbon monoxide, 9
  chlordiazepoxide, 73
  diazepam, 73, 207
  ethanol, 129
  fenfluramine, 207
  kanamycin, 45
  lead, 85, 93, 129, 193
  LSD-25, 45
  d-methylamphetamine, 73
  methylmercury, 15, 129
  methylmercury chloride, 163
  narcotic analgesics, 179
  nitrous oxide, 67
  organophosphate anticholinesterases, 179
  pentobarbital, 73
  pilocarpine, 45
  polybrominated biphenyl, 9
  prochlorperazine, 207
  propoxyphene, 207
  pyrithiamine, 175
  quinidine, 45
  scopolamine, 179
  thiamine, 175
  toluene, 67, 113
Drug-affected sensory threshold
  auditory threshold, 45
  kanamycin, 45
  LSD-25, 45
  pilocarpine, 45
  quinidine, 45
  trailwise tracking method, 45
  visual threshold, 45
Drug-toxicant interactions
  physiological and neurobehavioral alterations during development in lead exposed rats, 193

Environmental chemicals
  psychological test methods: sensitivity to long term chemical exposure at work, 157
Environmental contaminants
  auditory discrimination, 9
  carbon monoxide, 9
  discriminative behavior, 9
  ketones, 9
  polybrominated biphenyl, 9
Environmental Protection Agency
  behavioral toxicity, 213
  screening strategies, 213
  standardization of tests, 213
Environmentally significant neurotoxins
  axonopathy, 189
  cellular response, 189
  myelinopathy, 189
  neuronopathy, 189
Ethanol
  behavioral toxicology, 129
  crop dysfunction, 129
  lead, 129
  methylmercury, 129

- Experimental animal models
  - comparative behavioral toxicology, 33
  - hearing loss, 33
  - toxic effects on sensory systems, 33

- Fenfluramine
  - a preliminary test battery for the investigation of the behavioral teratology of selected psychotropic drugs, 207
- Fixed ratio
  - operant conditioning of infant monkeys for toxicity testing, 85
- Food additives
  - applied behavior analysis, 149
  - behavioral epidemiology, 149
  - food colors, 149
  - randomization tests, 149
  - time series, 149
- Food colors
  - applied behavior analysis, 149
  - behavioral epidemiology, 149
  - food additives, 149
  - randomization tests, 149
  - time series, 149

- Hearing loss
  - comparative behavioral toxicology, 33
  - toxic effects on sensory systems in experimental animal models, 33

- Inhaled substances
  - ammonia, 67
  - nitrous oxide, 67
  - reinforcing properties, 67
  - toluene, 67

- Kanamycin
  - auditory threshold, 45
  - drug affected sensory threshold changes, 45
  - trailwise tracking method, 45
  - visual threshold, 45
- Ketones
  - auditory discrimination, 9
  - discrimination behavior, 9
  - environmental contaminants, 9

- Lead
  - behavioral toxicology, 129
  - crop dysfunction, 129
  - ethanol, 129
  - methylmercury, 129
  - operant conditioning of infant monkeys for toxicity testing, 85
- Lead exposure
  - affective behavior, 93
  - behavioral toxicology, 93
  - learning, 93
  - pre- and post-natal exposure, 93
  - social interaction, 93
- Lead toxicity
  - behavioral development, 193
  - behavioral toxicology, 193
  - drug-toxicant interactions, 193
  - screening tests, 193
  - seizure development, 193
  - temperature regulation development, 193
  - visual evoked response development, 193
- Learning
  - effects of pre- and post-natal lead on affective behavior, 93
- Locomotion patterns
  - quantitative analysis of rat behavior patterns in a residential maze, 163
- Long term chemical exposure
  - behavioral effects, 157
  - carbon disulfide, 157
  - cognitive tests, 157
  - environmental chemicals, 157
  - perceptual motor tests, 157
  - sensitivity, 157
- LSD-25
  - auditory threshold, 45
  - drug-affected sensory threshold changes, 45
  - trailwise tracking method, 45
  - visual threshold, 45

- Measurement technique
  - motor activity, 53
  - toxicity testing, 53
- d-Methylamphetamine
  - behavioral assessment of risk-taking and psychophysical function, 73
  - behavioral toxicology, 73
- Methylmercury
  - behavioral toxicology, 129
  - crop dysfunction, 129
  - ethanol, 129
  - lead, 129
  - neurotoxicity, 15
  - visual fields, 15
  - visual thresholds, 15
- Methylmercury chloride
  - quantitative analysis of rat behavior patterns in a residential maze, 163
- Method
  - motor activity: a survey of methods with potential use in toxicity testing
  - operant conditioning of infant monkeys (*Macaca fascicularis*) for toxicity testing, 85
- Methodological problems
  - behavioral tolerance, 179
  - behavioral toxicology, 179
- Morphology
  - toxic distal axonopathy, 187
- Motor activity
  - measurement technique, 53
  - toxicity testing, 53
- Myelinopathy
  - cellular responses to neurotoxic compounds of environmental significance, 189

- Neonatal exposure
  - operant conditioning of infant monkeys for toxicity testing, 85
- Neurobehavioral effects
  - 3-acetyl pyridine, 175
  - experimental models of ataxia, 175
  - pyrithiamine, 175
  - thiamine deficiency, 175

Neurobehavioral screening procedures
  examples of validation of neurobehavioral tests, 137
  factors to be considered in screening, 137
  neurobehavioral toxicity, 137
Neurobehavioral tests
  examples of validation, 137
  factors to be considered in screening, 137
  neurobehavioral screening procedures, 137
  neurobehavioral toxicity, 137
Neurobehavioral toxicity
  examples of validation of neurobehavioral tests, 137
  factors to be considered in screening, 137
  neurobehavioral screening procedures, 137
Neuronopathy
  cellular responses to neurotoxic compounds of environmental significance, 189
Neurotoxicants
  sensory disturbances, 23
  somatosensory system, 23
  vibration sensitivity, 23
Neurotoxicity
  methylmercury, 15
  visual fields, 15
  visual thresholds, 15
Neurotoxins
  axonopathy, 189
  cellular response, 189
  environmental significance, 189
  myelinopathy, 189
  neuronopathy, 189
Nitrous oxide
  ammonia, 67
  reinforcing properties of inhaled substances, 67
  toluene, 67

Operant behavior
  effects of solvents on schedule-controlled behavior, 113
Operant conditioning
  activity, 85
  discrimination reversal, 85
  fixed ratio, 85
  lead, 85
  neonatal exposure, 85
  toxicity testing, 85
  visual discrimination, 85

Pentobarbital
  behavioral assessment of risk-taking and psychophysical function, 73
Perceptual motor tests
  psychological test methods: sensitivity to long term chemical exposure at work, 157
Performance
  acquisition, 105
  behavioral toxicity, 105
  serial position sequences, 105
Pharmacological toxicants
  behavioral assessment of risk-taking and psychophysical function, 73
Pilocarpine
  auditory threshold, 45
  drug-affected sensory threshold changes, 45
  trailwise tracking method, 45
  visual threshold, 45
Polybrominated biphenyl
  auditory discrimination, 9
  discrimination behavior, 9
  environmental contaminants, 9
Prochlorperazine
  a preliminary test battery for the investigation of the behavioral teratology of selected psychotropic drugs, 207
Propoxyphene
  a preliminary test battery for the investigation of the behavioral teratology of selected psychotropic drugs, 207
Psychological test methods
  behavioral effects, 157
  carbon disulfide, 157
  cognitive tests, 157
  environmental chemicals, 157
  perceptual motor tests, 157
  sensitivity to long term chemical exposure at work, 157
Psychophysical function
  amobarbital, 73
  behavioral toxicology, 73
  chlordiazepoxide, 73
  diazepam, 73
  d-methylamphetamine, 73
  pentobarbital, 73
Pyrithiamine
  comparison of neurobehavioral effects induced by various experimental models of ataxia, 175

Quantitative analysis
  activity, 163
  behavioral toxicology, 163
  locomotion patterns, 163
  methylmercury chloride, 163
  rat behavior patterns in a residential maze, 163
Quinidine
  auditory threshold, 45
  drug-affected sensory threshold changes, 45
  trailwise tracking method, 45
  visual threshold, 45

Randomization tests
  applied behavior analysis, 149
  behavioral epidemiology, 149
  food additives, 149
  food colors, 149
  time series, 149
Rat behavior patterns
  activity, 163
  behavioral toxicology, 163
  locomotion patterns, 163
  methylmercury chloride, 163
  quantitative analysis, 163
  residential maze, 163
Rate-dependent effects
  effects of solvents on schedule-controlled behavior, 113
Reinforcing properties
  ammonia, 67
  inhaled substances, 67
  nitrous oxide, 67
  toluene, 67
Residential maze
  activity, 163
  behavioral toxicology, 163
  locomotion patterns, 163
  methylmercury chloride, 163
  quantitative analysis of rat behavior patterns, 163

- Risk-taking
  - amobarbital, 73
  - auditory thresholds, 73
  - behavioral toxicology, 73
  - chlordiazepoxide, 73
  - diazepam, 73
  - d-methylamphetamine, 73
  - pentobarbital, 73
  - pharmacological toxicants, 73
  - psychophysical functions, 73

- Safety-testing
  - atropine, 119
  - behavioral toxicity, 119
  - caffeine, 119
  - schedule-controlled responding, 119
  - spontaneous motor activity, 119
- Schedule-controlled responding
  - atropine, 119
  - behavioral toxicity, 119
  - caffeine, 119
  - spontaneous motor activity, 119
  - testing for behavioral effects of agents, 119
- Screening factors
  - examples of validation of neurobehavioral tests, 137
  - neurobehavioral screening procedures, 137
  - neurobehavioral toxicity, 137
- Screening strategies
  - behavioral toxicity, 213
  - Environmental Protection Agency, 213
  - standardization of tests, 213
- Screening tests
  - physiological and neurobehavioral alterations during development in lead exposed rats, 193
- Seizure development
  - physiological and neurobehavioral alterations during development in lead exposed rats, 193
- Sensory disturbances
  - neurotoxicants, 23
  - somatosensory system, 23
  - vibration sensitivity, 23
- Sensory systems
  - comparative behavioral toxicology, 33
  - experimental animal models, 33
  - hearing loss, 33
  - toxic effects, 33
- Serial position sequences
  - acqusition, 105
  - behavioral toxicity, 105
  - performance, 105
- Social interaction
  - effects of pre- and post-natal lead on affective behavior, 93
- Solvents
  - chronic inhalation, 113
  - operant behavior, 113
  - rate-dependent effects, 113
  - temporal discrimination, 113
  - toluene, 113
- Somatosensory system
  - neurotoxicants, 23
  - sensory disturbances, 23
  - vibration sensitivity, 23
- Spontaneous motor activity
  - atropine, 119
  - behavioral toxicity, 119
  - caffeine, 119
  - schedule-controlled responding, 119
  - testing for behavioral effects of agents, 119
- Standardization of tests
  - behavioral toxicity, 213
  - screening strategies for the Environmental Protection Agency, 213
- Temperature regulation development
  - physiological and neurobehavioral alterations during development in lead exposed rats, 193
- Temporal discrimination
  - effects of solvents on schedule-controlled behavior, 113
- Thiamine deficiency
  - comparison of neurobehavioral effects induced by various experimental models of ataxia, 175
- Time series
  - applied behavior analysis, 149
  - behavioral epidemiology, 149
  - food additives, 149
  - food colors, 149
  - randomization tests, 149
- Toluene
  - ammonia, 67
  - effects of solvents on schedule-controlled behavior, 113
  - nitrous oxide, 67
  - reinforcing properties of inhaled substances, 67
- Toxic distal axonopathy
  - morphology, 187
- Toxic effects
  - comparative behavioral toxicology, 33
  - hearing loss, 33
  - sensory systems in experimental animal models, 33
- Toxicity testing
  - activity, 85
  - discrimination reversal, 85
  - fixed ratio, 85
  - lead, 85
  - measurement technique, 53
  - motor activity, 53
  - neonatal exposure, 85
  - operant conditioning, 85
  - visual discrimination, 85
- Trailwise tracking method
  - auditory threshold, 45
  - drug-affected sensory threshold changes, 45
  - kanamycin, 45
  - LSD-25, 45
  - pilocarpine, 45
  - quinidine, 45
  - visual threshold, 45

- Visual discrimination
  - operant conditioning of infant monkeys for toxicity testing, 85
- Visual evoked response development
  - physiological and neurobehavioral alterations during development in lead exposed rats, 193
- Visual fields
  - methylmercury, 15
  - neurotoxicity, 15
  - visual thresholds, 15
- Vibration sensitivity
  - neurotoxicants, 23
  - sensory disturbances, 23
  - somatosensory system, 23
- Visual threshold
  - auditory threshold, 45
  - drug-affected sensory threshold changes, 45
  - kanamycin, 45
  - LSD-25, 45
  - methylmercury, 15
  - neurotoxicity, 15
  - pilocarpine, 45
  - quinidine, 45
  - trailwise tracking method, 45
  - visual fields, 15